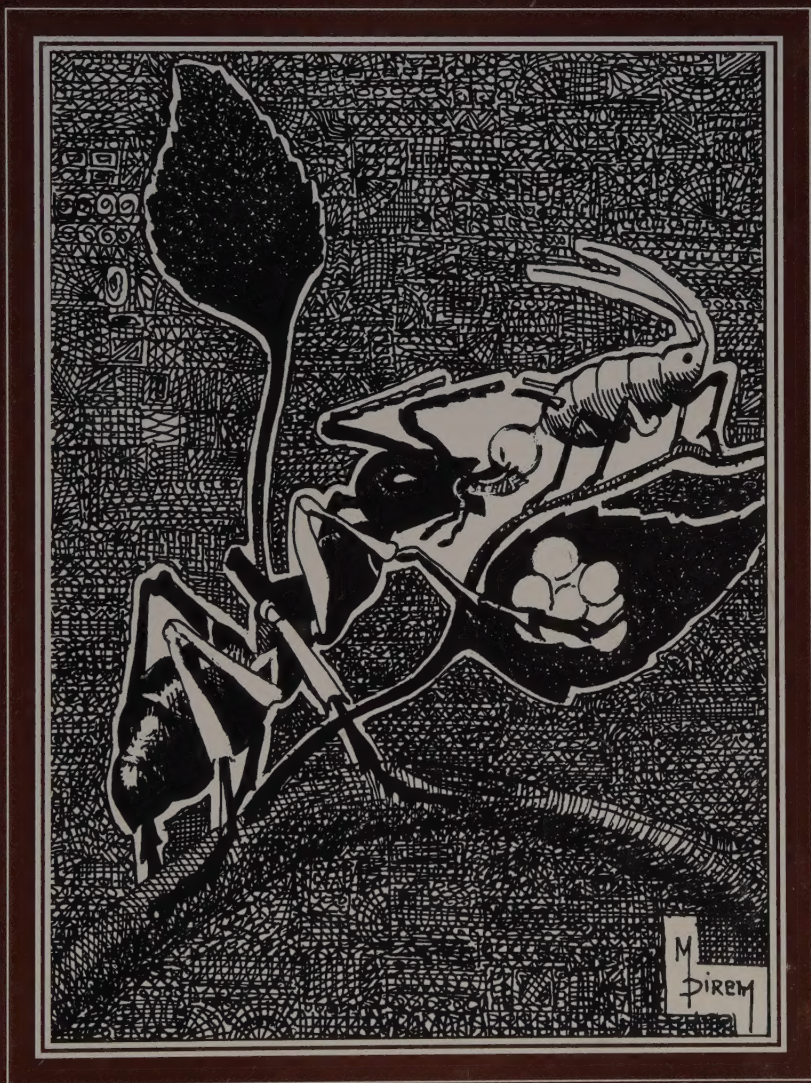


CHEMISTRY AND BIOLOGY OF SOCIAL INSECTS

Edited by
JÖRG EDER and HEINZ REMBOLD



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1987

CHEMISTRY AND BIOLOGY OF SOCIAL INSECTS

is a collection of more than 350 papers which were presented at the 10th International Congress of the International Union for the Study of Social Insects, IUSSI, in München 1986.

About 400 authors are presenting the latest developments in the fast growing area of research on social insects. Insect societies are attracting scientists from different fields of research.

To the classical - and still most important - areas of taxonomy, systematics, and morphology they add new ones in neurobiology, biochemistry, chemistry, physics, and sociology.

Recent data on interactions between individuals within and between social insects and plants, as well as the role of social insects in ecosystems are presented from their different aspects. The insights gained add to our understanding of life in a rapidly changing world. They will not only stimulate further interdisciplinary research in this fascinating field of social insects, but may also add to a better understanding of our human society.

The papers collected in *Chemistry and Biology of Social Insects* are exceptional from the standpoint of relevance to current knowledge. Together with its carefully edited author- and subject index, the book as a whole has much to offer to students, teachers, and researchers.

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**CHEMISTRY AND BIOLOGY
OF SOCIAL INSECTS**

JÖRG MEYER and HANS-JÜRGEN MEYER

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International Union for the Study of Social Insects

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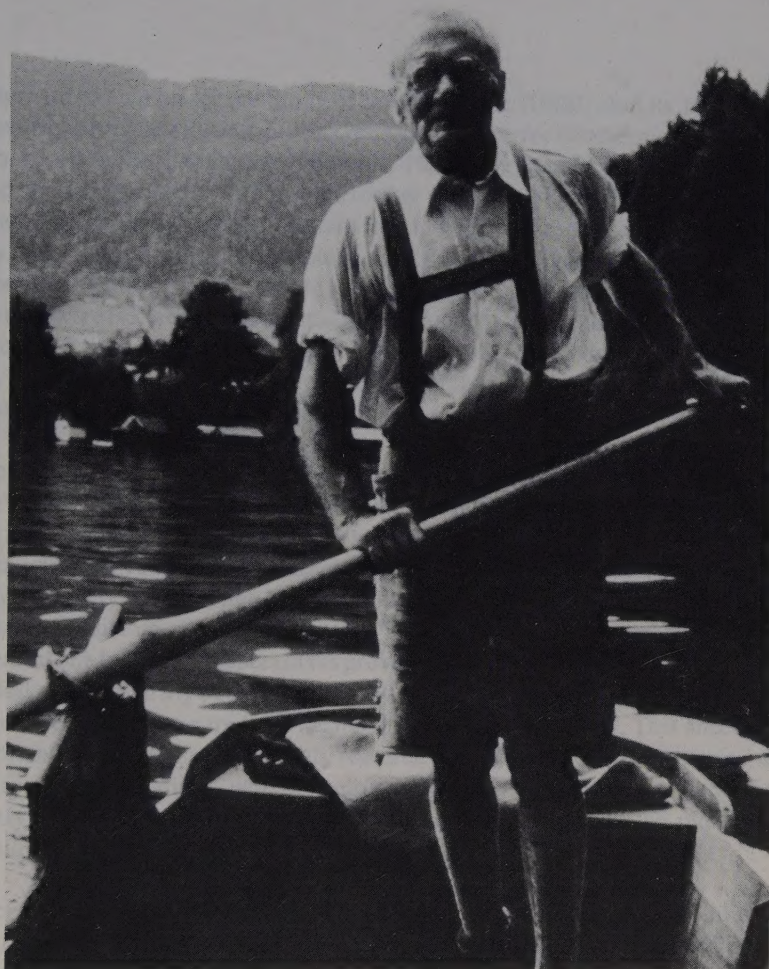
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FOREWORD

Following a request of the International Association of Agricultural Economists (IAAE) in 1981, the



Karl Ritter von Frisch
(1886-1982)

FOREWORD

Celebrating a centennial in memoriam Karl Ritter von Frisch (1886-1982)

The International Union for the Study of Social Insects, IUSSI, held its 10th International Congress from August 18-22 at the Max-Planck-Institute for Biochemistry which is located on the outskirts of the state capital of Bavaria. This center of basic biochemical research was founded and inaugurated in 1973 by the former president of the Max-Planck-Gesellschaft, Professor Adolf Butenandt. Through him it has, besides its research activities in molecular biology, also a long tradition in the field of bioactive natural products. In Butenandt's former MPI for Biochemistry, first in Tübingen, after 1956 in München, such important insect compounds were isolated and structurally elucidated as the ommochromes, the sex attractant bombycol and the moulting hormone ecdysone from *Bombyx mori*. A chemist for himself, he has, with this type of interdisciplinary research, become a pioneer of present insect biochemistry.

When in 1956 A. Butenandt and his institute came to München, Karl von Frisch had already retired from the university and his chair at the Institute of Zoology. However, he still took an interest also in that collaborative work which I had started at that time as the chemist, together with my colleague G. Hanser as the biologist, on honey bee caste formation. Butenandt one day told v. Frisch of our intention to rear honey bee larvae in an incubator and on an artificial diet up to their adult stage. We had no idea that we could make it and we experienced one interesting aspect of his proverbial scepticism. Von Frisch argued: tell your young folks that, from my point of view, they cannot succeed with this idea. A bee larva not only needs food, but also care from the nurse bees - but let them try. This anecdote touches an important aspect of interdisciplinary research - the adventure of overstepping one's own boundary of specialization - with the help of both sides, that of biology as well as that of chemistry. To demonstrate this was one of our intentions when we planned the 10th International Congress of IUSSI.

We celebrate Karl v. Frisch's 100th birthday also with this Proceedings Volume and it is Martin Lindauer who keynotes his merits as a pioneer in these modern fields of sensory physiology and experimental sociobiology. Karl von Frisch has been one of the pioneers who led us to the borders - and encouraged us also to cross them.

Heinz Rembold

President
10th Internat. Congr. IUSSI

PREFACE

CHEMISTRY AND BIOLOGY OF SOCIAL INSECTS is a collection of more than 350 papers which were presented in 18 plenary lectures, 26 symposia and the same number of poster sessions at the 10th International Congress of the International Union for the Study of Social Insects, IUSSI, in München 1986. In this volume, more than 400 authors are presenting the latest developments in the fast growing area of research on social insects.

Insect societies are attracting scientists from different fields of research. To the classical - and still most important - areas of taxonomy, systematics, and morphology they add new ones in neurobiology, biochemistry, chemistry, physics, and sociology. Recent data on interactions between individuals within and between insect societies, between social insects and plants, as well as the role of social insects in ecosystems are presented from their different aspects. The insights gained add to our understanding of life in a rapidly changing world. They will not only stimulate further interdisciplinary research in this fascinating field of social insects, but may also add to a better understanding of our human society.

With two opening addresses in memoriam Karl von Frisch the Proceedings of IUSSI 86 commemorate almost a century of entomology in honor of a scientist, whose investigations not only encompassed many basic aspects of zoology, but also popularised them in books which were translated into most of the living languages.

The papers collected in CHEMISTRY AND BIOLOGY OF SOCIAL INSECTS are exceptional from the standpoint of relevance to current knowledge. The book as a whole has much to offer to students, teachers, and researchers.

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The Editors

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OPENING ADDRESSES

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Caste Differentiation of the Honey Bee - Fourteen Years of Biochemical Research at Martinsried

Heinz Rembold

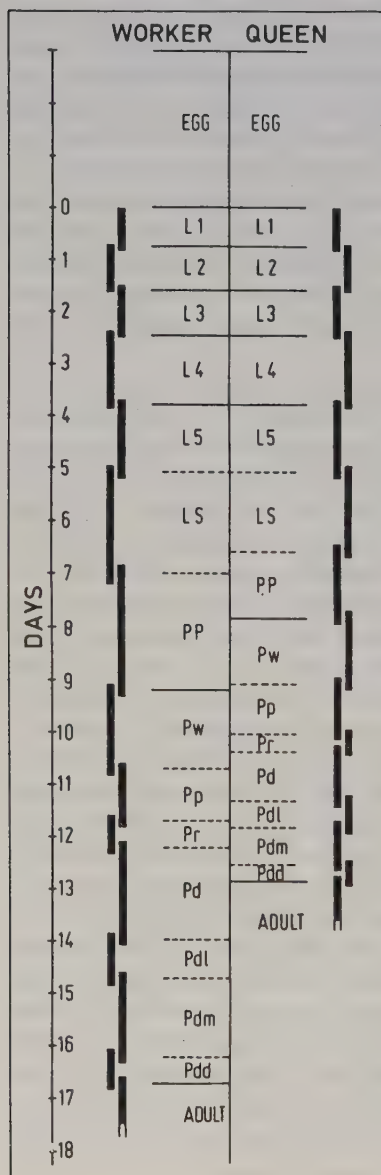
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It is a fascinating challenge for the biochemist to decode what the biologist calls structure, development, regulation, genetics, communication, and differentiation, in his own language of proteins, nucleic acids, hormones, pool size, and in relation to the molecular basis of gene expression and its control. There is a fundamental difference in how these two disciplines approach such problems. However, this difference is rapidly diminishing. Whereas in historical times the biologists used to follow a merely descriptive way in contemplating on nature and whereas, on the other hand, the organic - or now the bioorganic - chemists tried to define life in terms of chemical structures, correspondingly a new attitude towards life has developed with its beginning in the fifties. Since that time biology has assumed a central rôle within the sciences and biochemistry has taken a similar place within biology with the consequence that more complicated biological systems are now being studied and also on a more molecular genetic level than before.

One aspect of such a more biochemical view of a typically biological question, namely the control of caste formation in the honey bee, *Apis mellifera*, will be presented in the following. The chemical and biochemical facets in the development of this state forming insect have been a research topic of the Insect Biochemistry Unit at the Max-Planck-Institute from the very beginning here at Martinsried and over the last fourteen years. This biochemical model for a better understanding of differential morphogenesis and gene expression has come out not only to add to our understanding of queen and worker bee establishment but also has induced new ideas in the field of integrated pest control strategies. In other words - knowing more about the way which nature adopts to produce sterile female honey bees, also helps us to learn more about insects in general and as such even to control pest insects more efficiently. It was with this expectation, that the German Ministry for Research and Technology (BMFT) supported our studies on honey bee queen formation at the beginning of our work here at Martinsried. And it gives me great satisfaction to demonstrate some fifteen years later, that this argument finally proves to have been correct.

How can the honey bee colony manage the problem of producing three morphologically so different and functionally perfect individuals as the worker, the queen, and the drone? It has long been known that the males develop from unfertilized, haploid eggs whereas the reproductive female originates from fertilized eggs and the infertile worker bee as well. There is a sensitive control within the colony on queen production and when opening the hive, one finds only a few additional queens, most of them still in their cells or just newly hatched, none of them mated. There is only one egg-laying queen within the colony and all the other few thousand drones and about twenty to thirty thousand workers are her offspring. And there is an additional mystery - neither the virgin queens nor the drones seem to mate within the hive. What an enormous risk for an insect state and what precautions must be provided in case of an eventual loss of the only reproductive female, the queen!

There are many safety devices in order to minimize such a risk, as, for instance, the signals coming from the queen to inform the nestmates about her presence, i.e. the queen substance, or signals released by the workers, all controlling the harmony within a honey bee colony. And not the queen but the sterile workers finally decide upon the production of a new reproductive female. We can easily follow their decision after having removed the queen from the hive. Within hours the carefully balanced harmony disappears from the colony and a sound which is typical for queenless colonies can be heard. The wax producing bees start constructing queen cells on top of some selected cells containing the very young worker larvae, and these prospective queen larvae are then immediately and abundantly nourished with queen food.



This *Royal Jelly* can easily be collected in gram quantities from such a queenless colony. We have used up over the years more than fifty kilograms of this material, a fact which also demonstrates what a heavy meal must be presented to the growing larvae within the queen cell. This effect of queen rearing from a worker larva proves conclusively that in principle each female has the potential of becoming a reproductive, egg laying queen - at least during the early period of its development. This could also often be demonstrated by an artificial transfer of worker larvae into queen cells, and vice versa. What is the explanation for this sensitive period in honey bee morphogenesis and is there anything visible which could help us in finding the key, the *primum movens*, for this differentiation which finally creates two highly specialized individuals which are morphologically and functionally so different from each other, albeit having the identical set of genes?

In order to find a convincing answer, let us first consider the time period which is needed for imaginal development of a queen or of a worker. During the first three days of larval development which is identical with the sensitive phase of caste

Fig. 1. Duration of larval and pupal stages in worker and queen honey bees. The vertical bars show where the ranges of duration of adjacent stages overlap.

Abbreviations: L1 - L4 = 1st - 4th larval instar; L5 = 5th larval instar before sealing; LS = 5th larval instar after sealing; PP = prepupa; P = pupa; w = white eyes; p = pink eyes; d = dark brown eyes; dl = dark brown eyes, light pigmented thorax; dm = dark brown eyes, medium coloured thorax; dd = dark brown eyes, dark thorax (after Rembold, Kremer and Ulrich, 1980).

differentiation, both the prospective workers and queens are gaining the same body weight. This effect demonstrates that during the sensitive period of caste induction the worker larva is also sufficiently nourished. Mere starvation therefore does not induce development to a worker - or prevent queen differentiation. In the fourth larval instar of the worker, food quality, and again not its quantity, is drastically changed. The worker larvae are now fed a mixture of glandular secretion to which an ever increasing amount of pollen and nectar is added. The queen larvae, however, are continuously fed pure royal jelly. With this much more valuable food they now start growing faster than the workers.

Food change after the third larval instar of the female honey bee is a clear indicator of an effect which is finally responsible for caste differentiation. At the end of their feeding period, the queen larvae have finally doubled their weight as compared with the spinning worker larvae. However, here already it must be indicated, that by rearing the larvae artificially on a controlled diet, we end up with worker larvae which have reached the same weight as have the queens. It is not the amount of food offered to the larva and correspondingly the final weight, therefore, which decides upon the formation of queens or workers.

How does the difference in food quality after the third larval instar affect stages and as such duration of larval and pupal development? As shown in figure 1, the queen in its fifth, last larval instar speeds up with an increasingly short time needed for its larval and pupal development. This is an acceleration by about one third in comparison with worker development! When we look at the time table for the production of a worker and of a queen bee and when we compare it with the dramatic differences in growth rate occurring already during the fourth larval instar, we come to the following postulate which can be experimentally studied:

LARVAL FOOD QUALITY DETERMINES CASTE DIFFERENTIATION

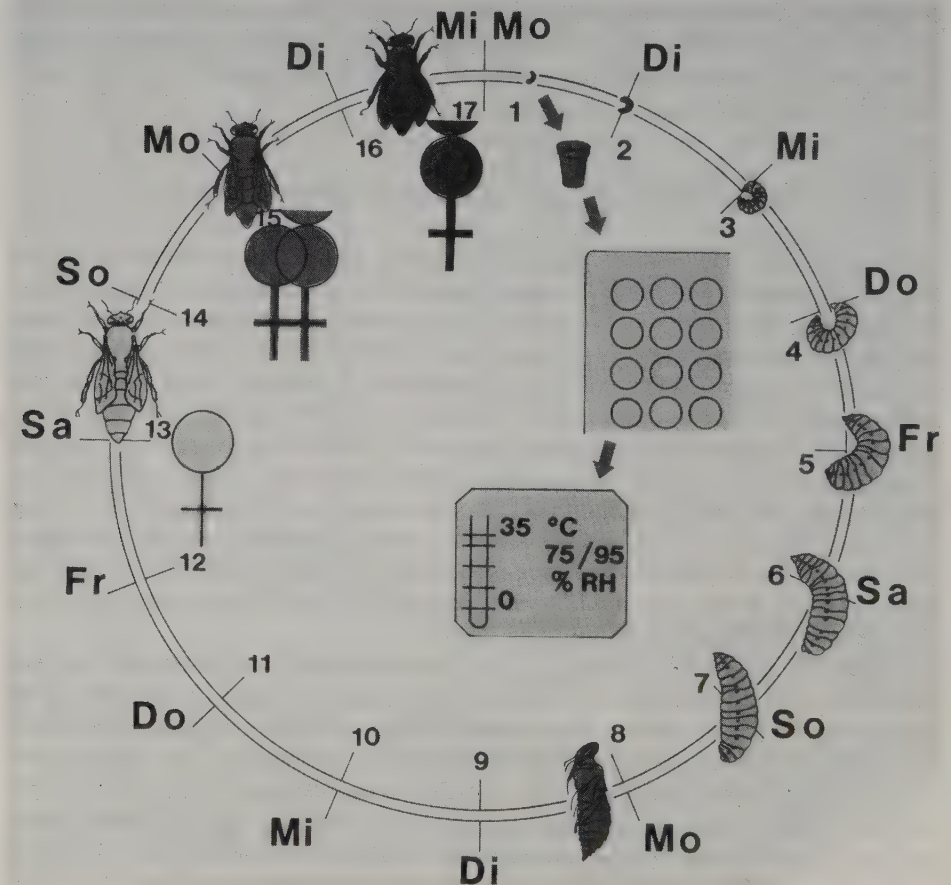
In other, more biochemical terms, this statement means that the genes, which are responsible for the caste-specific morphological expression, must be switched on only at the end of larval development. According to our understanding of genetic programming, this whole event must be under hormonal control. This seems to be clear from the fact that juvenile hormone I (JH I) induces queenlike morphological characteristics when topically applied to last instar worker larvae, as first demonstrated by WIRTZ and BEETSMA in 1972. We later found, that JH III is the *natural* hormone in the honey bee. It turned out to be much less active in its caste inducing capacity, however. This hormonal effect after topical application in an already determined larval stage directs our attention to the relation of food quality and activation of the endocrine system. However, before we can approach this question more in depth, we have to understand the relation of food quality and caste determination a little bit better. In other words - we want to understand the role which the nurse bees play in connection with feeding and caste establishment.

In order to reach clear results by any experimental approach, we first have to remove all the influences on our test larvae other than food quality. This can only be achieved by rearing them during their whole development in a carefully controlled environment other than the brood nest. This prerequisite also includes the age of the larva at beginning of the test. We place the newly hatched worker larva - it has a weight between 0.2 and 0.6 mg - from its brood cell into a plastic cup (most convenient are plastic thimbles or other disposable plastic material as used for microbiological purposes) on top of 0.1 ml of a defined food. Then these artificial brood cells are, usually in groups of 60 per test, placed into an incubator, the climate

of which is carefully maintained at 35°C and 95 % relative humidity. Twice a day we feed these larvae one drop of fresh food. At the third day of the test, excess food is carefully removed from each cell and new food added. A representative group of 10 larvae is also weighed at this occasion, and 15 hours later again, so arriving at important information about the average weight gain at the fourth larval instar. Near to the end of their feeding period the amount of food added must be carefully adapted to the feeding capacity of the last instar larva. If provided enough food, they reach a final weight of up to 300 mg-queens as well as workers - and then start spinning their cocoon. No food must be left over at this spinning period. The larva then passes the pharate and enters the pupal stage, when the test is transferred to an incubator which is kept at the lower relative humidity of 75 %.

Under our experimental conditions all these developmental steps follow the same time sequence as in the brood nest of a honey bee colony. This is schematically shown in figure 2. Such a result is surprising for several reasons. The larva is growing in the plastic thimble in

Fig. 2. Artificial rearing of honey bee larvae under controlled conditions in an incubator. The test starts on Monday, when a newly hatched worker larva is placed into a plastic cup on top of a defined diet. Groups of 60 are placed into a rack and fed twice a day one drop of food. After pupation, the test animals are transferred into another incubator and kept at lower relative humidity for further imaginal development (after REMBOLD and LACKNER, 1981).



too big a brood cell if compared with the natural one. In this plastic cell it further lies in a position which is completely different from the horizontal one of a worker and the vertical, downwards directed position of a queen cell. Gravity also does not control pupation as demonstrated by the fact that, under our test conditions, the pupae are found in their cups in all the positions which are physically possible. Also the time sequence which, according to figure 1, the two castes require for their development, follows this scheme, the queens hatching first, followed by the intercastes - which are animals with characteristics of a queen and of a worker as well, and which are usually not found in a honey bee colony - and finally the workers.

By comparing the many morphological caste differences, two - the mandibles and the basitarsus - came out to be so significantly different, that we selected six classes, one each for queens and workers which are morphologically identical with those collected from the colony, two with only minute differences from them and also found under natural conditions, and finally two with such animals which are real intercastes with mixed worker and queenlike characteristics. The experimentally grown queens can be artificially inseminated and then start laying eggs and establishing their own colony. With these results we primarily prove, that exclusively the food controls caste determination of the female honey bee. Even the intensive brood care by the nurse bees - after LINDAUER, each worker larva is visited during its development about 150 times and each queen larva even 1600 times - does not affect the result from our experimental brood care which corresponds to about 10 visits per larval development! From these results we conclude, that neither the nursing bees nor the position of a larva within the cell or within the colony - queen cells are usually found at the periphery of the brood nest-influences caste differentiation. A clear correlation between food quality and the number of queens having developed under our experimental conditions can be expected therefore.

Let me now illustrate the nutritional effects on caste differentiation with a few selected examples. As a basic food we may use a mixture of royal jelly, which was 1:1 diluted with water and supplemented with glucose and fructose to a final sugar concentration of 185 mg/g. Just for comparison - the average sugar content of unchanged royal jelly is at about 120 mg/g. With this test food and in a standard test with 60 larvae, 75 % of the first instar larvae became adults. However, most of them developed to workers and only 10 % became queens. If we now added to this basic food 1 % and in another test 2 % of yeast extract, a dramatic effect resulted. At the same survival rate we now came to a three- and resp. fivefold higher number of queens. Is there a "determinator" in the royal jelly, eventually also in the yeast extract, which stimulates queen production? We can try to get an answer from the following tests. We separated, by column chromatography, the yeast extract into two fractions, the first mainly containing the inorganic salts and the second the amino acids, each of which was added as an equivalent for 1 % yeast extract to the basic food. The result is surprising again. With an addition of the first fraction to our basic food we came to a food which dramatically reduced the number of adults to 50 %. Caste differentiation, however, was with 20 % still significantly higher than with the basic food alone. With the second fraction, survival was with 30 % even lower and queen differentiation was below the result obtained with the basic food. Recombination of both the fractions resulted in the same rate of survival and caste differentiation as when the same amount of original yeast extract had been added. It emerged from this study, that only an optimum nutritional balance can induce an optimum growth rate during a critical period of larval development. However, it cannot yet be concluded from these results, whether or not also a "determinator" is essential for caste determination, in addition to the nutritional balance.

For getting a clear answer about the two possibilities whether only nutritional balance or its combination with a determining compound controls caste differentiation we should use a fully synthetic diet, the composition of which is completely known and thus can be experimentally changed. However, whereas the low molecular compounds of royal jelly could be analyzed and quantified, we were unable to find any protein which would withstand the high acidity of royal jelly without denaturation. Keeping in mind, that the original queen larval food as collected from the brood cell must be 1 : 1 diluted with sugar solution so that the larvae can feed on it, we analyzed all the compounds found in the low molecular material and supplemented them by the same amount of altogether 50 synthetic compounds including amino acids, vitamins, nucleotides and inorganic salts. By using this trick, we ended up with a complete royal jelly, which, however, contained only 50 % of its high molecular weight material. We were now in a position to modify the composition of this semisynthetic diet and to follow its effect on queen formation.

Again, only a few examples from our extensive experimental screening can be given. We modulated the concentrations of one or more of the low molecular compounds and checked the effect on survival and determination when starting with the first instar worker larvae. The first example may demonstrate the effect of amino acids on caste differentiation. The amount of total amino acids was lowered in our semisynthetic diet to 0.5 g, all the other compounds remaining unchanged. The result was a 30 % queen formation. An increase of the amino acids to 0.75 and then to 1.0 g stimulated larval growth correspondingly. If now, in the latter food, the concentration of the nucleotides was doubled, we finally ended up with a determination rate of about 60 %. After doubling also the vitamin concentration in this experimental royal jelly, we reached a rate of queen determination which was near to 90 %. Among the animals classified as queens, 75 % were class 1 queens.

These data clearly demonstrate that an optimum nutritional balance is responsible for queen determination. Even the effect of the high molecular royal jelly fraction can now be understood through its lability and therefore with its becoming insoluble during extended storage of the jelly. The more indigestible this protein becomes for the larva, the more does it induce a nutritional imbalance. This also explains why a given royal jelly sample slowly reduces and finally even loses its determining activity: not a labile "queen determinator" gets lost, but its nutritional balance which is essential for the fast growing bee larva which for its whole larval period does not defecate and therefore cannot excrete most of its metabolites. Most critical seems to be the nitrogen balance and, correspondingly, the carbohydrate to nitrogen ratio. Consequently, either an increase of carbohydrate or a decrease of nitrogen containing compounds in the food strongly affects larval determination. In addition, the queen determining effect of such rare compounds as the nucleotides can be explained by some metabolic bottleneck situations which occur during a period of intensive larval growth. Keeping this sensitive nutritional power on queen determination in mind, we realize a situation for the fast growing honey bee larva which is fairly similar to that of a mammalian embryo in its maternal uterus. In both cases, temperature and nutrition have to be finely controlled in order to secure an optimal development.

Taking all these arguments together, we realize that there seems to exist a tight link between food quality and the endocrine system of the honey bee larva. If this is correct, we may ask which sensitive part of the hormone system is then under nutritional control and which are the candidates? It is common knowledge that the titers of ecdysone and juvenile hormone are under neurosecretory control. Those peptide hormones, which control their titers, are released from the neurosecretory cells of the *pars intercerebralis*, are transported through axons to the *corpora cardiaca* and are then released into the haemolymph. From

there they reach their target organs, the ecdysone producing prothorax glands and the corpora allata which release the JH.

It transpired to our surprise, that in the first instar honey bee larva the neurosecretory system of the brain is still in an embryonic or incomplete stage of development. The neurosecretory cells, especially, differentiate during further larval life in a caste-specific way, faster in queen larvae than in workers. In queen larvae, outgrowth of axons from the embryonic neurosecretory cells in the pars intercerebralis begins in the second instar and is completed at the end of third instar. In the worker larva, onset (third instar) and termination (fourth instar) of axonal outgrowth is one larval instar later. Stainable neurosecretory material is detectable in the late fifth instar queen larvae but not until the pupal stage of the worker. The caste- (and as such food-) specific growth and development of the neurosecretory system in the brain during larval development is therefore the *primum movens* in honey bee caste differentiation. This leads us to our second postulate:

HONEY BEE CASTE FORMATION IS TRIGGERED BY THE TIME REQUIRED FOR ACTIVATION AND MATURATION OF THE NEUROSECRETORY SYSTEM. INCREASING CONTINUOUSLY FROM QUEEN TO WORKER, THIS TIME IS SPECIFIC FOR THE RESP. CASTE OR INTERCASTE.

How does this caste-specific maturation of the neuroendocrine system affect the hormone producing glands? We studied this question by use of light-microscopic techniques. The corpora cardiaca are embryonic in both the castes until the end of third larval instar. In the fourth instar, which is a stage after the sensitive phase, they start growing and then to differentiate. At the end of this stage, and in the worker only in the last larval stage, labelled uridine is incorporated into this neurohaemal organ, thus demonstrating its metabolic activity.

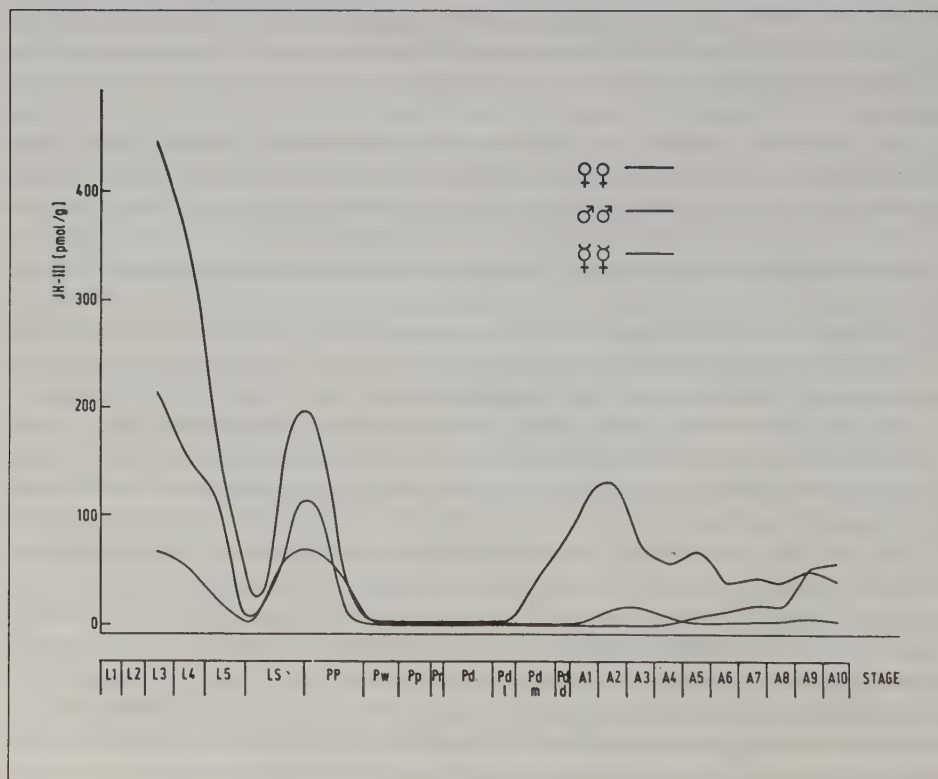
The retardation in worker larval development is also reflected in the development of the JH-producing corpora allata (CA). From the third instar onwards, the queen CA are always more voluminous than those of the corresponding worker stage. During larval development, the nuclei of the CA undergo several stages of endomitosis and again the queen larva gains a time advantage of about one instar. The caste- and growth-specific changes in volume and DNA synthesis seem to be connected with each other. This is shown by different levels of incorporation of tritiated thymidine, a DNA precursor, during the phase of condensed (no incorporation) and decondensed (incorporation) nuclei. This demonstrates that there are several steps of endomitosis and that the organ becomes mature in the last larval instar only.

Incorporation of labelled uridine into the ecdysone producing prothoracic glands is found at the second larval stage and at similar levels in both the castes. Labelling of the gland is fairly constant until the end of the third instar. It then decreases during the following larval periods and reaches a zero level at the end of fifth larval instar which indicates that synthesis of RNA now has ceased. The ecdysteroid titers correspondingly do not differ significantly between the castes. The hormone titer decreases steeply during the fifth larval instar and mainly in the pharate pupa from about 500 to a level near to 40 ng ecdysterone per gram of body weight. The hormone titer increases again, immediately before ecdysis to the pupa, and reaches a final and fairly constant concentration near to $1 \mu\text{g} \cdot \text{g}^{-1}$. As in the histological picture, there is again a clear time shift in the reactivation of ecdysterone synthesis, with a reversal period during the spinning phase in the queen and in the pharate worker pupa. Both the castes reach the same molting hormone titer soon after pupation.

Whereas the ecdysteroid titers do not show a dramatic caste-specific difference, this is true for the JH-titer curves which correspondingly play a dominant role in the whole process of caste formation. Before we could start a broad study of hormone titer modulation, we had to develop a highly sensitive GC-MS method which finally allowed us to quantify the juvenile hormone concentration in an individual honey bee larva. The result from a detailed JH III titer estimation during development of queens, workers, and drones is presented in figure 3.

During their imaginal development, the queen stages have a JH III titer more than twofold higher when compared with the respective drone and worker stage. Qualitatively, however, no great differences are seen between the two female castes or the males. In each case, the titers decrease from an initially high larval titer to a first minimum at the end of food uptake. Then another hormone peak builds up during the spinning stage which again decreases and reaches another titer minimum just at the end of the prepupal stage. Whereas during pupal development no hormone is detectable in both workers and drones, the queen pupa starts building up a fairly high JH III titer before its pupal - adult molt. In the workers, only a small peak is formed at the beginning of their adult stages, in the drones even only after their sexual maturation.

Fig. 3. Modulation of JH III titers of queens, workers, and drones of the honey bee, *Apis mellifera*, during imaginal development. In all stages, the queens keep the highest concentrations, followed by the drones with a characteristic delay in the adult stage and a peak building up around day 9. The worker stages always occupy the lowest titer values. For details of the developmental stages compare legend of figure 1 (after REMBOLD, 1986, and unpubl. results).



The quantitative caste-specific JH III titer differences reflect a fundamental process in the development of a reproductive (i.e. the queen) and a non reproductive (i.e. the worker). At stage LS/PP, the gonads are being resorbed in the fifth instar worker larva. The number of ovarioles is reduced from more than 300 in the queen to about 10 in the worker. It seems possible, that JH here, besides controlling larval - pupal molt, has an additional function, which is to protect an organ by acting as a maintenance hormone. In contrast to the queen, the JH peak in the last instar worker larva is not sufficient to protect the gonads from being degraded. Vitellogenesis and egg maturation then starts in the dark-eyed, melanizing queen pupa with all the consequences for morphological differences of both the female honey bee castes. This leads us to our third postulate:

A CASTE-SPECIFIC JUVENILE HORMONE TITER CONTROLS SEXUAL MATURATION OF THE HONEY BEE QUEEN

Morphogenesis is, in terms of molecular biology, the translation of DNA sequences, which represent the blueprint of developmental programs, into a protein sequence, which may then act as an enzyme and synthesize such structural elements as lipids or polysaccharides. With this reversible activation of genetic information in mind, and from the fact that ecdysone and JH titers are modulated during the last larval instar, a caste-specific modulation of protein synthesis can be expected which finally reflects the corresponding hormone titer modulations. One example for a caste-specific protein synthesis, that of the respiratory enzyme cytochrome c, has been studied by us from the beginning here at Martinsried. The protein was shown by OSANAI and REMBOLD (1968) to be present in the mitochondria of the worker larvae only in limiting amounts. This may be the explanation for the well known physiological difference in oxygen consumption between worker and queen larvae which was first described by MELAMPY and WILLIS in 1939. By use of a highly sensitive viro-immunoassay, we estimated the cytochrome c content of worker and queen larvae. It resulted from these studies, that the product of respiratory quotient (R) times the logarithm of larval cytochrome c content, divided by the body weight (W), yields a caste-specific constant (K):

$$K = \frac{R \cdot \log \text{cyt } c}{W}$$

The queen-specific value K_q is about 3 and that of the worker (K_w) about 0.8, according to EDER, KREMER and REMBOLD (1983). Interesting enough, from a series of experimental larvae which had been reared in vitro, some had the queen value, some that of the worker, and some were in between, indicating that they were intercastes.

We now can sum up all the steps which finally induce caste formation during honey bee larval development. If we admit that life for itself is miraculous, nothing of an additional mystery is then left over for the process of honey bee caste formation. Development of the female larvae follows the general scheme of a hymenopteran insect with only one crucial exception. A change in the hormonal information transfer from the neurosecretory system to the peripheral hormone glands is used in order to trigger a caste-specific developmental program. As discussed already, a successive sequence in the expression of a caste-specific morphogenesis has been demonstrated. Only under the most optimal condition, which is reflected by an intensive weight gain in the fourth instar larva, is the queen-forming response of the female honey bee larva switched on during the third larval instar. By a difference in

food quality in the colony, or by feeding an imbalanced diet in vitro, the activation of the controlling system in the larval brain is delayed by more than one instar in the worker. The following step, therefore, is a time difference in the initiation of hormonal regulation. During this period both worker and queen larvae start with a phase of intensive growth which is not under the control of JH directly. The third step is a caste-specific maturation of the peripheral hormone glands, especially the CA. Only in the last larval instar is caste induction during the third larval stage reflected by a modulated hormone synthesis, primarily of JH. The fourth important step is then a queen-specific JH III peak which coincides with the maintenance of the female gonads and with a breakdown of most of the ovarioles in the worker larva. With this important event, the caste-specific programming comes to its end. In a last step, the queen and worker developmental programs are expressed during the pupal phase.

Much of this clear chain of events is still purely a model for other social insects. The establishment of intercastes may lead to a better understanding of the complicated mechanisms which control caste formation in such social insects as ants or termites. As already mentioned at the beginning, the basic mechanism of inducing some sort of sterility in an insect by interference with its hormonal regulation of growth and development, is another attractive result from our study on the basis of honey bee caste formation. We consequently initiated a search for plant metabolites which interfere with insect morphogenesis.

By use of the *Epilachna varivestis* bioassay we tested a great variety of indigenous and tropical plants for their potency in inhibiting insect growth and metamorphosis. There are indeed quite a few plants, the extracts of which interfere with the molting process of *Epilachna* larvae. Our most advanced results in this field came from a study of the tropical neem tree, *Azadirachta indica*, from the seeds of which we isolated a series of structurally related triterpenoids and which we now combine as the group of azadirachtins.

All these compounds exert the same physiological effect: whereas non-toxic for mammals, they derange the hormone system not only of *Epilachna*, but of practically all insect species studied so far. In ppm concentration azadirachtin inhibits postembryonic development, induces morphological aberration, high mortality and a considerable reduction in egg laying. Detailed studies on the mode of azadirachtin action were undertaken in our laboratory with *Locusta migratoria*. After a single injection of 10 μ g azadirachtin into female adults, the ecdysone titer was lowered to trace amounts or the peak was shifted on the time axis. Modification and suppression of the ecdysteroid titer by azadirachtin is closely correlated with morphogenetic effects. The long lasting decrease in ecdysteroid titers in treated larvae is paralleled by the same effect on juvenile hormone. A single injection of 2 μ g azadirachtin per gram completely abolishes the JH III peak in the female adult with the consequence that no vitellogenin synthesis and no egg maturation is initiated.

The effect of the azadirachtin group on the two morphogenetic hormones can be interpreted as an interference with the neuroendocrine system quite similar to the situation in the female honey bee. In both cases a reduction or even blockade of prothoracotropic (PTTH) and allatotropic (ATH) hormone release inhibits the peripheral control of hormone synthesis with the consequence of a reduction of ecdysone and juvenile hormone titers and consequently of morphogenesis and egg production in the reproductive insect. Histological staining of the neurosecretory material in the brain of azadirachtin-treated locusts indeed revealed a remarkably high accumulation of stainable material in the corpus cardiacum. We assume that the release of the trophic hormones from this organ into the haemolymph is reduced or even inhibited by azadirachtin and that this is the first step in its mode of action.

A more target specific control of insect growth and reproduction is required. The problems of environmental hazards are often associated with our conventional broad spectrum insecticides. An increasing number of insecticide-resistant pests stimulates the search for new control strategies. Application of pheromones or hormone mimics are one possibility. When discussing such alternative plant protection strategies, we must recognize, that these methods have become available only after extensive basic studies in insect physiology and biochemistry. A better understanding of the relationships between the insect and its host plant, especially of their chemical basis, can also help the plant breeder in establishing more insect resistant crop varieties. These are only a few examples which demonstrate, how much has to be and also can be done in the no-man's land between basic and applied biology. During a relatively long period of complete reliance on conventional pesticides, the understanding of fundamental processes involved in our interaction with insects has not been appreciated by the scientist. The translation of biological principles into chemical signals and their incorporation into applied programs therefore often fails in default of biological understanding on the chemist's as well as on the biologist's side. One addition to this frontier-crossing adventure has also come from our fourteen years of biochemical research on caste differentiation at Martinsried.

Acknowledgement

A long list of inspired coworkers results from this work. Only a few have been mentioned, although thanks are due to each of them. Instead of such a list, a few typical publications will be cited as a source for additional information about our work on honey bee caste differentiation.

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Karl von Frisch, a Pioneer in Sensory Physiology and Experimental Sociobiology

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We human beings roam across vales and hills, meadows and woods, and delight in the fragrant carpet of flowers in their colourful magnificence, in the chirping of crickets and the song of birds. But who would imagine that our experience is only a tiny fraction of the real environment? Physicists have shown, e.g., that the electromagnetic spectrum extends from the long wavelengths of radio waves via the infrared and ultraviolet to the short wavelengths of x-rays and gamma rays. The human eye perceives only the minute section between 400 and 800 nm - approximately a billionth part of the spectrum! We know today, that for other animals important information exists in the field of ultrasound to which our human ear is deaf. Biochemists have analyzed the structure of numerous molecules by which many animals are attracted, or from which they shy away, but to which our nose is not sensitive in any way. It was a major achievement in this century when Karl von Frisch, the pioneer of modern sensory physiology and behavioral research, presented to us the means for the discovery of such unknown worlds. It is easier to understand the work of this passionate biologist if one looks into the intimate circumstances of his family life, where his love of nature was already fostered at an early age. Born in the Josephstädter Strasse, a corner protected from the metropolitan noise of Vienna, he turned his room into a menagerie during his early grammar school days already. His diary lists as companions: 9 different kinds of mammals, 16 species of birds, 26 different reptiles and amphibians, 27 kinds of fish, and 45 species of invertebrates. Later on he wrote, *"I shall never forget my mother's patient tolerance towards those sometimes obnoxious companions"*.

From his earliest childhood he showed the curiosity characteristic of the future zoologist, in that he not only described the morphology and behavior of an animal, but also asked questions concerning the causal relationships which govern, e.g., the function of the vital processes. His sharp power of observation may be testified by a letter written to his mother when he was only nine years old:

"Dear Mamma, just consider, my salamanders which I brought back from Kolta, laid 30 eggs yesterday afternoon, and from last night till today 31 eggs, each one distinctly separated on a different leaf of a water plant, which they later put together in this way: (A drawing is included).

Many regards from Your Karl".

Decisive new ideas and considerable encouragement for his later profession as an enthusiastic researcher in the field of comparative animal physiology came from his uncle Sigmund Exner, Professor of Physiology at the University of Vienna. After his doctoral thesis on color changes in fish, v. Frisch turned to the subject of sensory physiology and became the pioneer of this new zoological discipline.

In 1912, a colony of bees was accommodated at the summer residence of the von Frisch family, which was situated at Brunnwinkl, near St. Gilgen in the Salzkammergut. This colony had been acquired because v. Frisch wanted to challenge a thesis of the famous

ophthalmologist C. von Hess (of Munich) who had stated that all invertebrates were completely colorblind. V. Frisch asked himself why the colorful patterns of meadows did exist, if it were not to act as "Wirtshausschilder", or "innsigns", i.e., to lead the bees towards the sources of nectar. Introducing food training experiments as a new method, v. Frisch began to test the bees' sensory perception, and especially the problem of how bees take their bearings within their environment and how they communicate.

With an extremely simple equipment, he tested the bees in a series of well planned experiments. A dish of sugar water was offered to attract foraging bees to a table, the dish being placed on a piece of blue cardboard so that the bees saw the color "Blue" as they approached the table and fed on the sugar water. Using a fine brush and shellac paint, the bees were individually marked as they sucked the sugar water, so that the behavior of the forager bees could be observed from behind the glass panes of an "observation hive" after their return. It is the very simplicity of the methods and tools used, that characterizes the genius behind many of v. Frisch's experiments. It should be emphasized that v. Frisch was always very critical about his own experimentation. This attitude is illustrated by a personal remark he once made to me during my doctoral thesis: *"Denken Sie immer, um Ihren Futtertisch stehen 5 Beobachter, die versuchen, alles zu kritisieren, was Sie mit Ihren Bienen tun"*. (Always imagine that there are 5 observers standing around your experimental table, and that they are trying to criticize everything you do with your bees). The particular caution he exerted on his color sense experiments is well known among bee researchers. To ensure that the bees could really distinguish color, and not simply differentiated between various shades of grey, the blue cardboard he used was surrounded by cardboard pieces of the same size ranging from white through various shades of grey until black. The position of the card was constantly changed to avoid the possibility that the bees were being trained to the card location. Close attention was required to avoid traces of scent being deposited on the blue cardboard when the bees alighted, and for this reason, new, clean cardboards were always used. Well founded and convincing results came only after painstaking and detailed work, as illustrated by the researcher's own words: *"The fruits I could reap were sparsely placed between many great disappointments"*. With success of course, came great joy, highlighted by an event in 1914, when v. Frisch presented at the congress of zoologists in Freiburg *"A demonstration of experiments to prove color perception in totally color blind animals"*. The color training of the bees was demonstrated in the open air, and it is reported that during the experiment the bees alighted obediently on the empty blue cardboard. When they found it empty, one bee searched in the near vicinity and landed on the blue tie of one of the onlookers! This was enough to convince even sceptical congress members that bees were indeed capable of color perception.

I should like to point out here a particular feature which was characteristic for all of v. Frisch's experimental work: he always used his discoveries and especially those concerning the bee dance, as a basis and starting point for new ideas and experimental designs. This very strategy enabled him to build interdisciplinary bridges also to ecological problems, and, looking back at his research work, we can say that it was the basis of a new discipline which now is called ethoecology. This is demonstrated in an impressive way by his experiments on the color sense of bees. Initially, it was found that the bee's eye is insensitive to red, and that red is in fact seen as a shade of grey. Since the visible spectrum of the bee is shifted into the short-wave-length domain, ultraviolet also becomes an important color for these animals. This finding raised the question of how far this perception of U.V.-light was connected with the biology of the bee. Forty years later, the problem was solved by a student of v. Frisch - K. Daumer. Now we know that the "apparently" white and

yellow blossoms of flowers are seen by the bee as an impressive U.V.-pattern that shows the way to the nectar-guide. Combined with yellow on the petals of flowers, this U.V. results in the so-called "bee-purple", a brilliant "color" for the bee's eye. Every lover of nature must surely feel a sense of amazement when he or she realizes the close relationship between the characteristics of the bee eye and the color of flowers, and that this relationship is a "symbiosis" that has evolved over many millions of years.

Over a period of time, more complicated and intricate questions began to be approached with regard to the bee's perception of forms or its recognition of patterns, and each time a new wealth of information was discovered. Today, much of this knowledge fills text books and conference reports in the fields of sensory physiology, neurobiology, and ecology - an astonishing fact when we realize that the basic work for all this knowledge on bees was performed only 74 years ago at Brunnwinkl on the Wolfgangsee.

Much progress was also made in other fields of sensory physiology. At that time, it was not clear where the bee's sense organs for smelling were located. An American scientist had put forward a theory saying that the whole chitin shell was interspersed with olfactory cells, but v. Frisch established beyond all reasonable doubt that the antennae were in fact the base of the sense of smell. He was fascinated with the idea that the tactile sensory system of the antennae, together with the olfactory system, would enable the bee to have a "spatial sense of smell", or "Räumliches Riechen" as he called it. This concept has achieved high priority in the field of neurobiology with regard to the "multimodal" processing of information. Within later investigations on chemoreceptors, Schneider and Boeckh discovered receptors that were "specialists" for certain scents to which our human noses are completely insensitive. Among these scents certain categories are of special importance to bees, as, e.g., the scent of the Nasanoff glands (Sterzelduft), which attracts returning bees to the hive, or the scent of the queen's mandibular gland through which she communicates with the drones on her nuptial flight. This research has fundamentally contributed to the knowledge which is the base of biological pest control, a promising approach to fight pest insects threatening wood and crops of fruit and cereals.

After the difficult years of the Nazi regime, when v. Frisch was under the threat of dismissal from employment, the end of World War II brought the highlight of v. Frisch's life. Although his first communication concerning the language of bees was published as early as 1920, the total information content of the bee's dance could be fully explained only 25 years later. It was already known that a successful forager bee, returning to the hive with a full honey stomach, performs a "round dance" on the honey comb with excited mincing steps, and that this dance announces the presence of food in the nearby vicinity. The scent of flowers carried home in the nectar and on the body hairs gives information regarding the species of flower that has been visited. The phenomenon of one bee communicating with other foraging bees, presenting them, within the hive, precise information on a food source distant from the hive, was certainly exciting enough. However, further investigations carried out at Brunnwinkl in 1945, led v. Frisch to assume that the dance of a bee conveys additional information indicating also direction and distance of the food source from the hive. The first surprise encountered was that the "round dance" changed to a "tailwagging dance" if the food table in his experiments was gradually moved to a distance of more than 50 m from the hive. V. Frisch concluded that the rhythm of the dance is related to distance, and that an increase in the distance between food source and hive results in a slower tailwagging during the dance. After this first discovery, deciphering the code which translates distance into rhythm was the next problem. For a food source 100 m from the hive, v. Frisch recorded 10 tailwagging runs per 1/4 minute, for 200 m the number of runs was reduced to 8 per 1/4 minute, for 500 m

to 6 per 1/4 minute, whilst for 1000 m only 4 runs per 1/4 minute were observed. Several years of tedious work were required to obtain these results. The investigations were frequently interrupted by changes of weather; additional difficulties arose from forager bees failing to show up, or from interference by foreign bees. Apart from being able to indicate distances, the bees are also able to accurately indicate the direction of a food source. How can this be achieved in the case of, e.g., a very small target situated 8 km from the hive? For a food source that was kept constant, it was observed that the direction of the tailwagging run on a vertically placed honey comb changed from hour to hour, and in such a way that the direction was directly proportional to the changing position of the sun as it moved across the sky. In other words, on its flight the bee memorizes the angle between the actual position of the sun and the course of her flight, this angle then being expressed as vectors of the gravitational field during the dance in the dark hive. This is an astonishing achievement of the bee brain, since the dance angle to be communicated has to be transferred from an optical perception to a gravitational one. The information represented in the dance on the comb has been shown to be equally valid for all bee species studied to date. Thus, for example, a tailwagging run straight up means "you must fly directly towards the sun", whilst a tailwagging run straight down means "the food source is directly opposite to the sun", and a run which is inclined 60° to the left of the vertical position indicates that the food source lies 60° to the left of the sun. It is quite clear, therefore, that the angle between flight course and sun is directly proportional to the field of gravity.

In the light of this remarkable discovery, problems which had not been taken into account thus far, were now approached and elucidated. The biggest surprise was that bees continued to use the sun as a reference point during their dances even when the sun was hidden behind clouds or large structures such as a rock. As long as there is a small patch of blue sky visible, the bees are able to utilize the pattern of polarized light to fix the position of the sun. I was fortunate enough to witness the crucial experiment that demonstrated this ability. When a polaroid sheet held above the head of a dancing bee was rotated in various directions, the angle of the tailwagging also changed accordingly. After this discovery, scientists hurried to search for the part of the eye that was able to detect polarized light. It is well established now that the molecules of the optical pigment are oriented in parallel within the microvilli of the optical cells, and that this arrangement is the basis of "dichroitic absorption".

Using the sun as a compass does, of course, present the bee with a major problem. Unlike a magnetic compass, which constantly indicates one direction, the orientation system of the bee must account for the changing position of the sun in the sky during each day and throughout the year. It was already known that bees have an excellent sense of time, but a convincing explanation of the bee's ability to account for the changes in the position of the sun was still not available. A blue print for such an "experimentum crucis" had been existing in v. Frisch's desk for many years, but he always hesitated in giving effect to such an investigation. *"People will think we have gone crazy if we attempt to ascribe such a complicated system of orientation to the bees"*. We kept, however, encouraging each other and finally ventured into the experiment. A bee hive was transferred from its native habitat to an alien locality, and the same afternoon a small group of bees was trained to fly to a food source located 200 m south of the hive. We expected that the bees, on their way to the target, would memorize distinctive features of the environment, and that they would also make allowance for the position of the sun, i.e. that they would fly to the left of the sun in order to reach their destination. During the night, the hive and the south-trained bees were transferred to a nonfamiliar environment. The next morning, four feeding tables were set up, one at each point of the magnetic compass and each one at a distance of 200 m from the

mother hive. The question that was being asked to the test was: Would the bees fly southward (as they had been trained), or would they choose the eastern feeding site, i.e. the feeding site located at the same angle to the sun as the site where they had found food the day before. To be quite honest, we did not expect very much of an answer at all, because we were afraid that the bees would return to the hive as soon as they had left the flight hole and noticed a strange environment.

Our apprehensions didn't come true, however, the south-trained bees flew directly to the true south and thus proved that the sun was indeed the perfect point of orientation. Today, it is well known that many other groups of animals as, e.g., ants, crabs, birds, fish, salamanders, tortoises etc. use this sun orientation system as well.

I was privileged to spend 15 summers with Karl von Frisch, conducting these experiments and many other investigations. Throughout this time, there constantly arose new questions, such as: How do bees circumnavigate a projecting rock? How does a bee deal with a wind that is blowing at an oblique angle to the direction of flight? Is there a special code in the bee dance to indicate whether the target is located up- or downwards? How relevant are the features of the landscape as compared with the sun compass? One observation was puzzling and could not be explained at this early stage: Performing their dances, the bees make small mistakes which are not accidental, but display a regular sequence in the course of the day. For 10 years, I continued to investigate this problem with my colleague H. Martin, and we finally arrived at the conclusion that dance parameters indicating the direction of flight are obviously influenced by the magnetic field of the earth. Nowadays, biomagnetism is a very up-to-date field of research. It is well known that many animals use the earth's magnetic field to orientate themselves within their environment, and that diurnal fluctuations of the magnetic field are also used as a time cue. Nothing is still known, however, about the perception mechanisms involved. New problems are still arising, and I would like to conclude this chapter with Karl von Frisch's own words:

*"The bees are a magic well,
The more you draw from it,
The more there is to be drawn."*

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1.1 Systematics

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SYMPOSIUM

Biosystematics of the Ant Tribe *Leptothoracini*

Organizer: Alfred Buschinger

Introduction

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The myrmicine tribe Leptothoracini is a group of ant species with an extraordinarily high number of interesting traits. Generally they have small colonies with only a few hundred adult specimens, living in often well-defined nesting sites. Collecting and studying complete societies therefore are not too difficult, and for most species practicable methods for maintaining numerous colonies in the laboratory have been developed. A considerable number of species even can be bred in artificial conditions over generations, a precondition for genetical studies.

Most sociobiological phenomena known from ants in general can be observed also in the leptothoracines: Monogyny, polygyny, functional monogyny, budding, polydomy, claustral and non-claustral colony foundation, sexual behavior, biorhythms, various recruitment mechanisms, slavery and inquilinism, aberrant male and female polymorphism and so on.

Much of the contemporary field and laboratory work, however, is complicated because of the obsolete systematics of the group both in the species and supraspecific levels. Recent studies of several working groups have revealed a large body of new informations which, when suitably compiled, might become the basis for a new, biologically as well as morphologically founded system of the group, representing the true phylogenetical relationships.

With this aim in mind a number of specialists, working with leptothoracines, have contributed their data to the symposium, and discussed the usefulness of a systematic rearrangement of the tribe as is prepared by Buschinger and Francoeur (see abstract BUSCHINGER). Biochemical methods (DOUWES and STILLE, HEINZE) obviously are important new tools for elucidating relationships of morphologically similar species, but also of higher categories. The results are matching well with conceptions derived from morphological and behavioral studies.

A rich variety of reproductive isolation mechanisms is described for the subgenus Myrafant (PLATEAUX). However, several species pairs are poorly isolated, with crossbreeding being possible in laboratory conditions. Some intermediate forms found in the field may originate from such hybridizations.

A contribution of HERBERS refers to seasonal changes in polydomy, and thus population structure of a North American species. This might be an important aspect in studies on polygyny and related subjects in quite a number of species.

Three lectures are dealing with social parasitism in leptothoracines. For the slavemaker genus Chalepoxenus a synonymization of most described species seems appropriate, since hybridization between all the investigated ones was obtained in the laboratory, morphological differences are small, karyotypes are identical, and colony foundation and slave raiding behaviors are essentially the same.

In Epimyrma (JESSEN) an evolutionary transition from species with truly dulotic life patterns through several intermediate stages to finally workerless forms has been demonstrated. This evolution is accompanied by a remarkable change from swarming to intranidal mating and inbreeding. Several species of the genus have to be synonymized, and Myrmoxenus apparently represents but an Epimyrma with a different number of antennal segments.

BOURKE and FRANKS finally are discussing the phylogenetic origin of the particularly rich variety of social parasites among the Leptothoracini. A sympatric speciation of the parasites from their hosts appears more likely than the traditional assumption of an allopatric speciation and subsequent parasitism between the closely related daughter species.

Both the lectures and the final discussion have revealed that great progress has been achieved with respect to a better understanding of the phylogenetical relationships within the tribe. The proposal to establish two subtribes, one including the present subgenus Myrafant together with its parasitic genera, and the other mainly comprising the subgenus Leptothorax and the genera parasitizing it, was generally approved of the participants.

The Use of Enzyme Electrophoresis in *Leptothorax* Classification

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In the attempt to bring taxonomic order in the Leptothoracine ants the use of electrophoretic data should be considered. In this study we are mainly concerned with the subgenus *Myrafant*, which contains the majority of the Leptothoracini species. The taxonomy of this subgenus is very confused, and therefor we have not been able to identify some of the species examined. Our aim is to see if genetic variation as revealed by enzyme analysis can be used to separate species. Most species in this study are represented by 5 or more colonies.

MATERIAL AND METHODS

Despite the small size of these ants individual workers can be analysed for at least 12 enzymes. Of the about 20 enzymes tested 5 loci were found to give scorable bands - phosphoglucose isomerase (PGI), malate dehydrogenase (MDH-1, MDH-2), isocitrate dehydrogenase (IDH), and phosphoglucomutase (PGM). The electrophoretic method is that of Selander et al. (1971) except for MDH-2, which showed best resolution on the buffer system of Clayton and Tretiak (1972). The electromorph variants of PGI, MDH, and IDH could be interpreted as allozymes, whereas the genetic basis of the variation in PGM is unclear (Fig. 1).

RESULTS

Interspecific variation is found at all 5 loci and most species are monomorphic or almost so (Fig. 2). PGI, IDH, and PGM show a high and approximately equal degree of variation, but considering the fact that many of the PGI variants are represented by rare alleles in some species

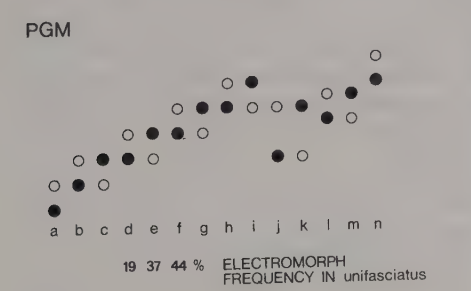


Fig. 1. Variation in PGM (14 electromorphs, a to n) and, as an example, the variation in *unifasciatus*. Solid = strong, open = weak band.

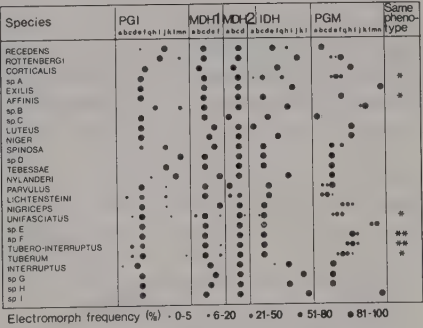


Fig. 2. Variation at 5 loci in *T. recedens* and 25 *Myrafant* spp.

IDH and PGM are more efficient in discriminating between species (Fig. 2). In all 5 loci together all but 6 species have unique phenotypes. Within the morphologically uniform tuborum-unifasciatus group (nigriceps to tuborum) 2 species pairs with more or less identical phenotypes occur, 1 pair (tuborum-unifasciatus) having the same phenotype as affinis and sp. A (remotely similar to affinis) (Fig. 2).

Although not being the primary goal of this study it is tempting to use the enzyme data for an analysis of the affinities between species. For this purpose we have included data from Leptothorax (s. str.) and some social parasites of Myrafant. Based on Nei's index of genetic identity of species (Nei, 1971) a WPGMA clustering (Sneath and Sokal, 1973) yielded the phenogram in Fig. 3. The Leptothorax (s. str.) spp. form a distinct group, while Temnothorax, Chalepoxenus, Myrmoxenus, and Epimyrm show more or less close affinities to Myrafant. This is in accordance with Buschinger's suggestion, based on morphology and biology, that the main division is between Leptothorax (s. str.) + related genera and Myrafant + Temnothorax + their parasites (Buschinger, pers. comm.) and also fits the pattern in isoelectric focusing (Heinze, pers. comm.). The isolated position of corticalis is interesting (Fig. 3).

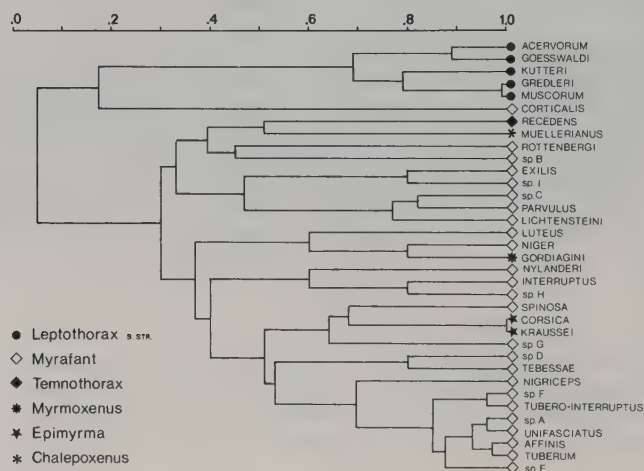


Fig. 3. Phenogram based on WPGMA cluster analysis of Nei's index of genetic identity of 35 Leptothoracini species.

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The Application of Electrophoretical Data on Species Differentiation in the Ant Tribe *Leptothoracini*

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Electrophoretic methods are widely acknowledged to be of some value to the systematics of insects, and keys for the determination of species based on allozyme data have been published in different genera (Berlocher, 1984). In ants species complexes such as *Iridomyrmex* "purpureus" (Halliday, 1975), *Aphaenogaster* "rudis" (Crozier, 1977) and *Rhytidoponera* "impressa" (Ward, 1980) have been dissolved into clusters of sibling species by analysis of enzyme polymorphism.

In the ant tribe *Leptothoracini* the systematic position of many species and genera is not fixed yet, and data in addition to morphological, ecological and karyological ones are badly needed to help rearrange this group of ants.

The present study is concerned with patterns of electrophoretically detectable intra- and interspecific variation in the ant tribe *Leptothoracini*, obtained by ultrathin-layer IEF of pupae.

MATERIALS AND METHODS

Colonies of different *Leptothoracini* were collected in North America, North Africa and Europe by Buschinger and coworkers during the past five years. Isoelectric focusing was performed on ultrathin polyacrylamide gels (0.2 to 0.3 mm). pH ranges were 3.5 to 9.5, 4 to 8 and 4 to 6. Whole worker, female and male pupae were crushed in 30 µl each of a solution containing 20 % glycerol and 2 % bromthymol blue, out of which 15 µl were applied to the gel and focused for 5000 volt-hours. Enzymes were visualized using standard histochemical stains.

RESULTS

Esterase-, MDH-, IDH- and SOD-stains of electrofocused *Leptothoracini* pupae homogenate give reproducible results in all species studied by now. No differences were found between patterns of pupae from, newly collected colonies and those bred in the laboratory for several breeding cycles, nor any variation due to different diets, such as using cockroaches or mealworms only as protein source. Unspecific esterase stains with 1-naphthyl acetate and Fast RED TR show complex patterns with 9 to 17 distinguishable bands in each species, out of which 4 to 6 are very intense. Other stains yield only 2 to 8 bands.

Esterase patterns remain constant in all major bands during the whole developmental cycle of pupae, with only minor changes after the inset of pigmentation. Adult ants show much less esterase activity than pupae. No differences were found between esterase patterns of worker and female pupae. Best results with least variation between different pupae of the same colony can be obtained by focusing homogenates of wholly unpigmented pupae or those with only slightly pigmented eyes.

All species studied by now (6 *Leptothorax* s.str. = *Mychothorax* Ruszky, 10 *Myrafant*, 3 *Epimyrmica*, 2 *Harpagoxenus*, 1 *Chalepoxenus*) show species-specific esterase patterns with no or little intraspecific variation. In all *Leptothorax* s.str. and both *Harpagoxenus sublaevis* and *H. canadensis* esterase patterns consist of four to five major bands and one or two allozymes of one locus with a pI of about 5.

The comparison of esterase patterns from 50 colonies of *L. "muscorum"* from Canada (12 populations in Quebec and 1 in the Rocky Mts.) and 15 colonies of European *L. muscorum* (1 population in Sweden and 3 in Germany) indicates, that the Canadian *L. "muscorum"* does not belong to the same species as the European *L. muscorum*, but instead consists of at least two sympatric species, which show only slight differences in morphology and behaviour. Similar results have been obtained by several other enzyme stains. Esterase patterns of *Myrafant* species are less uniform than that of *Leptothorax* s.str., but they all show a double band with pI of less than 5, which is absent in other genera and subgenera.

Esterase and other enzyme stains of *Harpagoxenus sublaevis* and *H. canadensis* indicate - whereas based on too few enzymes yet to apply any statistical method - a close relationship to *Leptothorax* s.str., but not to *Myrafant*, thus supporting ecologically-based conclusions drawn by Buschinger (1981).

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Reproductive Isolation in Ants of the Genus *Leptothorax*, Subgenus *Myrafant*

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The main event in the formation of a new species is its reproductive isolation. The social character of ants produces additional modalities of reproductive isolation. The small *Myrafant* ants are easily reared in tubular glass nests with a drinking tube and a dry extremity. The distance between the brood and the drinking tube is accorded to the hygro-preferendum of the species. The success of swarming depends on temperature and light.

A pregamic isolation may use a spatial ecological way when the species are separated by their biotopes, or a climatic ecological way when the species swarm over different thresholds of temperature. The pregamic isolation is strongly realized by different horaries of swarming (figure 1): The evening and the morning species can't cross. A same group of related species contains evening and morning ones. Therefore, the swarming in the evening or the morning depends on recent genetic characters. A mechanical isolation is possible between small queens *tubero-interruptus* and large males *unifasciatus*. An ethological isolation by pheromones seems to lack between the species of a same group: the mixed swarming of *parvulus* and *lichtensteini* seems to give panmictic results. This resembles the close related female pheromones of *L. goesswaldi*, *L. kutteri* and *D. pacis* (Buschinger, 1975). In return, the

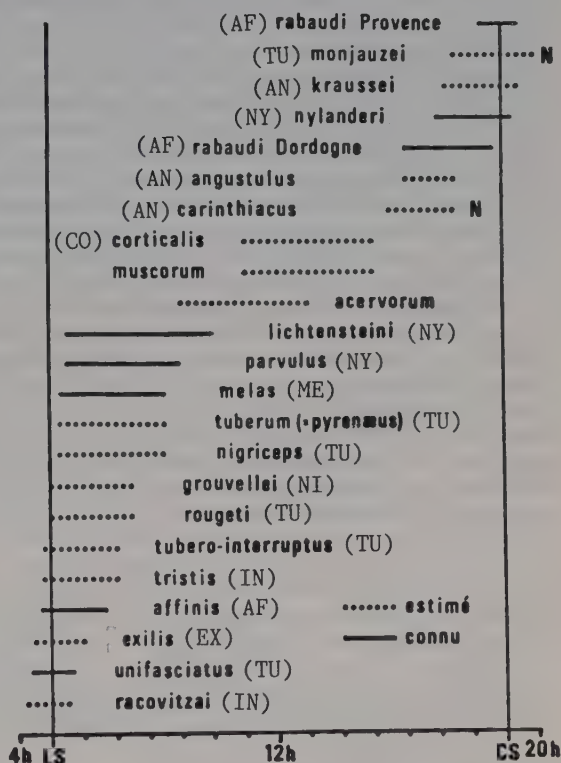


Figure 1: Horaries of swarming of 20 *Myrafant* and 2 *Leptothorax*. CS = sunset; LS = sunrise; connu = measured in laboratory, estimé = observed without measure of light; N = field observations (Bernard, 1957; Cagniant, 1968). (NY) = group *nylanderii*, (IN) = g. *interruptus*, (AF) = g. *tubum*, (CO) = g. *corticalis*, (EX) = g. *exilis*, (AN) = g. *angustulus*, (ME) = g. *melas*, (NI) = g. *niger*.

odour of females of a given group don't seem stimulating for males of another one, as shown by the failure of some crossings (nylanderi-rabaudi, etc.) or by the difficult mating of some species (lichtensteini-affinis, etc.).

A postgamic isolation is realized by hybrid weakness, or difficulty to produce hybrid queens, or hybrid aggressivity. Hybrid weakness is observed to foundations of unifasciatus queens mated by tubero-interruptus males, and in the progeny of one lichtensteini queen mated by affinis males. A difficulty to produce hybrid queens is observed in hybrid societies of unifasciatus queens mated by rougeti males. Meanwhile, in the same group, Seifert (1984) described field hybrid queens of nigriceps and unifasciatus.

Hybrid aggressivity is realized in more than 40 hybrid societies of parvulus and lichtensteini, in which the hybrid workers are strong, very good nurses and aggressive towards each other. The young hybrid queens are aggressive and attacked like workers. The hybrid societies lack cohesiveness and the larger ones are decimated during winter.

Hybrid workers, reared from larvae by nylanderi workers, fight with each other but not with nylanderi, as do hybrid workers reared by parvulus or lichtensteini; parvulus workers, reared from larvae by hybrids, suffer a slight aggressiveness from hybrids; lichtensteini workers, reared by hybrids, show a delayed aggressiveness (after overwintering) towards hybrids (which are the strongest). Therefore hybrids are more aggressive than the other ants and their aggressiveness seems to be stimulated by their equals. A first hypothesis implies an irregularity in the system of recognition between hybrid workers by discordant dominancies. A second hypothesis implies a heterosis giving an excessive concentration of pheromones of recognition.

The genetic exchange between different species of social insects may be stopped not only by the reproductive behaviour, but also by disturbances in the hybrid society. Sociality changes the relations between conspecific populations, which have to be more consistent between them than if they were non-social. Thus, in the case of aggressiveness between hybrids, the social condition leads to the isolation of the two species. In the absence of society, hybrids would take an advantage which might increase their fertility and reduce the two groups to the subspecific stage.

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Population Structure in *Leptothorax longispinosus*

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The facultatively polygynous ant, *Leptothorax longispinosus*, occurs throughout eastern North America in locally dense aggregations. Intense study of this species in three sites (West Virginia, New York, and Vermont) shows geographic variation in several aspects of their biology. The pattern of polygyny differs significantly among sites such that where nests are denser, multiple-queening is less common (Herbers 1986a). In addition, differences in nesting cavity type and frequency of social parasitism by *Harpagoxenus americanus* exist among sites; however, these factors appear to be unrelated to variation in queen numbers (Herbers 1986b). Rather, the variation between sites can best be explained as a differential response to nest site availability (Herbers 1986a).

There are also seasonal shifts in population structure within a site. Widespread collection of nests in New York and Vermont show that in summer nests are denser and have fewer queens per nest than in winter (Herbers 1986a). Moreover, as the summer progresses, nests initially spread out spatially and then later become more closely situated (Herbers 1985).

These results are best explained by a seasonal cycle in polydomy. Entire colonies coalesce for overwintering, perhaps to enhance survivorship (Herbers 1985b). In early spring, the ants become active, start to explore their surroundings, engage in aggressive interactions with neighbors, and undergo fission (Herbers and Tucker 1987). Thus polydomy ensues when an entire colony breaks up into several nesting aggregations. Despite physical segregation, phenomena such as worker movement and brood exchange allow colony subunits to maintain contact (Alloway et al 1982, Herbers and Tucker 1987). Over the growing season, nests continue to move about spatially and occupy additional nesting cavities, until alates are reared, in mid-July. By late August nesting aggregations have started to coalesce for overwintering, and by October the seasonal polydomy cycle has been completed.

Given this cycle of polydomy, it is difficult to determine colony boundaries in nature. We are using several approaches to identify nest fragments of entire colonies: 1) spatial analysis of excavated plots. Nests are patchily distributed within a site; clearly nests that are close together are more likely to be members of the same colony than are those separated by large distances. Analysis of nest density and statistical description of patchiness are a first step towards determining colony boundaries. 2) aggression tests among near neighbors. Work on kin recognition in this group shows that aggression between non-nestmates may be related to colony identity (Stuart 1985). Compatability tests conducted between nests closely situated in nature will yield information on potential colony

identity of nest fragments. 3) analysis of genetic relatedness. Electrophoretic examination of enzyme activity will provide the data by which to calculate coefficients of relatedness, both within- and between- nests (Crozier and Pamilo 1982). Nests that are fragments of larger colony units should share more alleles and those alleles should occur in similar frequency than nests originating from separate colonies. Thus, by using a variety of approaches, we will be able to ascertain which colonies are most likely to split up during spring conditions, how many fragments are produced per colony, and the duration by which colony-mates are separated in space.

This fluidity of population structure is not restricted to Leptothorax longispinosus. In the north temperate forests where our studies have occurred, other species are polydomous as well, including M. punctiventris, L. alienus, and T. caespitum. Moreover, nest migration and concomitant changes in spatial structuring of the community have been demonstrated (Herbers 1985). Thus in this habitat the prevailing concept of ant colonies stable in time and space is not generally valid.

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Evolution of Social Parasites in Leptothoracine Ants

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The ant tribe Leptothoracini is extremely rich in social parasites (Buschinger 1981). These ants therefore offer special opportunities to explore biosystematic aspects of Hymenopteran social parasitism. Here we consider two important topics in this field: the biological significance of Emery's rule, and the radiation of socially parasitic species subsequent to their original evolution.

Emery's rule states that the closest phylogenetic relatives of Hymenopteran social parasites are often their hosts (Wilson 1971). This observation may simply reflect the fact that social parasites must have a similar biology to their hosts. But this explanation only predicts a relatively close relationship of parasite and host, not the precise one-to-one phylogenetic correspondence that appears to exist in many taxa. In these cases Emery's rule arguably reflects a much more intriguing phenomenon - sympatric speciation of parasites from their hosts.

This suggestion is not original. Buschinger (1965) proposed that the workerless inquiline Leptothorax kutteri, which apart from being smaller is morphologically extremely like its host L. acervorum, could have originated when queens, genetically incapable of worker production, arose which survived by infiltrating conspecific colonies (a tactic for which they were pre-adapted by L. acervorum's habit of polygyny and queen adoption), and parasitically producing sexuals. Reproductive isolation of such forms would have resulted in a species like L. kutteri. We suggest that the hypothesis that intraspecific parasitism can lead to interspecific parasitism by a species generating its own parasite now has additional support from both Leptothoracines and other ants, and deserves further investigation.

Outside Leptothoracines support for this hypothesis comes from the existence of large and small queens (macrogyne and microgyne) with different biologies within polygynous species of Myrmica ants. In two cases microgyne have recently been shown (Elmes 1978) or are almost certain (Pearson and Child 1980) to be separate workerless inquiline species. In addition, a trend exists in the degree of microgyne specialization as social parasites within species of Myrmica (Pearson 1981). It seems unparsimonious to explain such cases, in which there is clear intergradation of intra- and inter-specific differences, as the result of allopatric speciation and secondary sympatry.

Microgyne may also occur in Leptothorax longispinosus. Franks (unpublished observations) found queen size to be bimodally distributed in each of two widely separated populations. Significantly, the closely related L. curvispinosus has a workerless inquiline parasite L. minutissimus which is morphologically a microgyne and apparently descended from its host (Buschinger 1981).

We now consider the radiation of social parasite species once they have arisen. Social parasites can undoubtedly give rise to other social parasites by allopatric speciation, as in other organisms, for

example if the common range of a host-parasite pair is divided by an isolating barrier. A succession of such events would result in a monophyletic assemblage of parasites coupled to a monophyletic assemblage of hosts. Such a pattern appears in the socially parasitic bumble bees Psithyrus and their hosts (Pamilo et al 1981). However, organisms which live in intimate host-specific associations with other organisms are also prone to an additional mode of speciation - sympatric speciation by the formation of races with different hosts (White 1978). This process would result in a monophyletic parasitic assemblage whose members exploit species not necessarily closely related to each other. The monophyletic Leptothoracine genus of social parasites Epimyrma, whose hosts include Leptothorax and Temnothorax (Buschinger 1981), could have evolved by this process.

Moreover, in the Leptothoracine slave-maker Harpagoxenus sublaevis allozyme analysis suggests that ants in a population where colonies occur with two slave species, are genetically differentiated by host (van der Have and Bourke, unpublished observations). This is preliminary evidence for host race formation.

In conclusion, sympatric speciation could be of particular importance in explaining two notable biosystematic patterns in Leptothoracines and other Hymenopteran social parasites. First it could help explain the extremely close phylogenetic relationship that often exists between parasite and host, and second monophyletic parasite assemblages associated with polyphyletic assemblages of hosts. Electrophoretic allozyme analysis (see Pearson and Child 1980 and Pamilo et al 1981), and behavioural studies of intraspecific parasitism, host specificity and host race formation, could be used to further test these ideas.

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Biosystematics of the Slavemaking Ant Genus *Chalepoxenus*

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The genus *Chalepoxenus* presently comprises 6 described species: *C. muellerianus* (Finzi 1921) (= *C. gribodoi* Menozzi 1922, (KUTTER 1973)), *C. insubricus* Kutter 1950, *C. siliensis* Kutter 1973, *C. kutteri* Cagniant 1973, *C. tramieri* Cagniant 1983 and finally *Leonomyrma spinosa* Arnoldi 1968. The latter was recently transferred into the genus *Chalepoxenus* by BUSCHINGER (in press).

During the last 65 years numerous *Chalepoxenus* populations have been detected in nearly all countries around the Mediterranean, especially in Southern Europe, all of them living as social parasites together with *Leptothorax* species belonging to the subgenera *Myrafant* M.R. Smith 1950 and *Temnothorax* Mayr 1861.

In arena experiments we could prove *C. muellerianus* to be an obligatorily dulotic ant (EHRHARDT 1982). Meanwhile the biology of the other species was studied, and we tried to elucidate the relationships between some of the species in order to get informations on their taxonomical position.

MATERIALS AND METHODS

We collected numerous *Chalepoxenus* colonies from the type localities of most species and from a lot of additional localities in Southern Europe, and many colonies of the various host species. Only *C. tramieri* from North Africa and *C. spinosus* from the Ural river/Russia could not yet be investigated.

Experiments on slave raiding and colony foundation were performed in plastic arenas with a thin plaster floor (EHRHARDT 1982). Mating behavior was observed in plastic flight cages (30x15x20 cm) in natural light conditions.

Beyond this karyotype analyses (method described by IMAI et al. 1977) and comparative morphological studies of some representative *Chalepoxenus* populations were done.

RESULTS

Recent behavioral studies revealed that presumably all *Chalepoxenus* species are true slavemakers. The biology of all species as far as yet examined is almost identical. This holds true especially for slave raiding, mating and colony foundation behavior.

Chalepoxenus conducts well organized slave raids comprising the four typical elements: scouting, recruitment of nestmates by tandem running, fighting by using the sting, and brood transportation: all brood is carried back to the

slavemakers' colony.

Sexuals of all *Chalepoxenus* species copulate in a kind of swarm flight. In the cages males and females often assemble in one corner towards the light forming a "swarm", in which single copulations happen.

Chalepoxenus founds new colonies in a typically dulotic way by force. The fecundated young female kills by stinging all the adults of a small host species colony, or drives them away, and occupies the remaining brood.

The great biological accordance of all *Chalepoxenus* forms is confirmed by the results of some experiments on interspecific correlations. Slave raids of colonies with a mixed stock of *Chalepoxenus* workers take an absolutely normal course with some mixed *Chalepoxenus* tandems etc., indicating that the behavioral patterns of different species are just exchangeable. This holds true on sexual behavior too. Mating between sexuals of all species is possible. Crossbred females produce workers, females and males, revealing that there exists no genetical barrier.

Arena experiments on host specificity prove *Chalepoxenus* not to be inflexibly fixed on the natural host species. In laboratory all *Chalepoxenus* species are able to attack also some host species of the other *Chalepoxenus* forms during slave raiding or colony foundation.

Morphology and karyotypes seem to be rather uniform within the genus *Chalepoxenus*. All species examined have an identical karyotype of $1n=12$. Morphological studies show some more or less distinct variations between and also within certain populations but they are not suitable for species identification. Clear morphological differences exist only between *C. kutteri* and the remaining forms. Also *C. spinosus* is marked by some morphological peculiarities (BUSCHINGER in press).

Summarizing the results of our investigations we suppose, that *Chalepoxenus* represents only one polytypical species with various populations, which due to geographical and ecological separation have reached different levels of speciation.

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Biosystematic Revision of the Parasitic Ant Genus *Epimyrma*

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The myrmicine genus *Epimyrma* Emery comprises 11 described species. They inhabit central and southern Europe and North Africa. They are all parasites on species of the genus *Leptothorax* (subgenus *Myrafant* and *Temnothorax*).

Little was known about the biology of this parasitic genus and fundamental systematic problems existed, e.g. the discrimination of the three species *E. kraussei*, *E. vandeli*, and *E. foreli* (Kutter 1973). As a conclusion of our morphological and biological studies on this genus we synonymize *E.v.* and *E.f.* with *E.k.* (Buschinger et al. in press).

Besides this the dulotic ant *Myrmoxenus gordiagini* and the genus *Epimyrma* show great similarities in their morphology, biology, and karyotype. A monophyletic origin of both genera is probable (Buschinger et al. 1983, Fischer, unpubl.). We suggest to transfer *gordiagini* into the genus *Epimyrma*.

MATERIAL AND METHODS

Numerous colonies of the various *Epimyrma* species were collected. We studied their population structures in the field and in the laboratory, their mode of colony foundation, slave-raiding and mating behavior. In addition crossbreeding experiments were carried out. Besides this investigations were made on the morphology and karyotype.

RESULTS

In the genus *Epimyrma* we have an evolutionary trend from active dulosis to degenerate dulosis which ends up in absolute workerlessness. Main differences in the population structures therefore are due to the number of ♀♀. The slavemakers produce numerous ♀♀ (*E. ravouxii* (= *E. goesswaldi*, Buschinger 1982), *E. algeriana*, *E. stumperi*, *M. gordiagini*), whereas in the degenerate slavemakers the production of ♀♀ is reduced (*E. kraussei*, incl. *E.v.* and *E.f.*). In *E. corsica* and *E.n.sp.* (Greece) no ♀♀ are produced (Buschinger & Winter, 1983, 1985; Buschinger et al. 1983, Buschinger et al. in press).

Within the genus *Epimyrma* two types of reproductive strategies occur: 1. The sexuals are swarming, colony foundation takes place in fall directly after swarming, the production of sexuals is balanced (*E. ravouxii*, *E. stumperi*, *M. gordiagini*); 2. The sexuals mate in the nest,

the production of ♂♂ is reduced, the mated ♀♀ remain in the nest over winter and leave it for colony foundation in spring (*E. bernardi*, *E. kraussei* (incl. *E.v.* and *E.f.*), *E. corsica*, *E.n.sp.* (Greece) (Buschinger et al. 1983, Winter & Buschinger 1983, Buschinger & Winter 1985, Buschinger et al. in press). In *E. algeriana* the sexuals also mate within the nest, the colony foundation presumably occurs in fall.

Mode of colony foundation and slave-raiding are essentially alike in all *Epimyrma* species tested (incl. *M. gordiagini*) (Winter 1979, Buschinger et al. 1983, Winter & Buschinger 1983, Buschinger et al. in press.).

All 7 *Epimyrma* species tested (incl. *M.g.*) have the same karyotype of $n = 10$ chromosomes. No other leptothoracine species tested showed this karyotype (Fischer, unpubl.).

Crossbreeding experiments have been successful with various populations of *E.k.* (incl. *E.v.* and *E.f.*) and between distinct good species, like *E.k.* with *E. bernardi* and *E. corsica* (Buschinger et al. in press).

As morphological comparisons of *E.k.*-populations (incl. *E.v.* and *E.f.*) show, there are no reliable morphological differences between the populations, that would support the existence of three different species (Buschinger et al. in press).

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Biological Arguments for a Systematic Rearrangement of the Ant Tribe *Leptothoracini*

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The actual system of leptothoracines, with the genus *Leptothorax* subdivided into several subgenera (*Leptothorax*, *Myrafant*, *Temnothorax* etc.), and some separate genera of parasitic species (*Chalepoxenus*, *Doronomyrmex*, *Epimyrma*, *Harpagoxenus*, *Myrmoxenus* etc.), is not satisfactory. Biological data are provided demonstrating that there exists a close relationship between subgenus *Leptothorax* and the genera *Formicoxenus*, *Doronomyrmex*, *Harpagoxenus* on the one hand, and between the subgenera *Myrafant* and *Temnothorax* and the genera *Epimyrma*, *Chalepoxenus* and *Myrmoxenus* on the other hand. These data include larval instars, sexual behavior, ecological needs, and host specificity of parasitic species. As far as investigated, the "*Leptothorax* group" has 4 larval instars, the "*Myrafant* group" only 3, in most "*L. group*"-species the females exhibit a stationary sexual calling behavior whereas the "*M. group*"-species make mating flights, "*L. group*"-species need only a short hibernation of about 6 weeks, and the parasitic genera always depend upon host species of the group to which they themselves do belong. A revised system of the *Leptothoracini* should regard these phylogenetically important biological features. They correspond with morphological characters also requiring a rearrangement of the tribe.

Taxonomic Revision of the Ant Genus *Formicoxenus* (*Formicidae*, *Hymenoptera*)

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Among the socially parasitic ants, the guest ants are representing a particular mode of coexistence with their hosts. They are living within their own nest chambers which are found in the nest material or nest walls of the host species. There they care for their own brood, and they depend from the hosts only in that the guest ant $\delta\delta$ are begging food from them. The guest ants up till recently were attributed to three leptothoracine genera, Leptothorax, Symmyrmica, and Formicoxenus.

Morphological studies including measurements, dissections of male and female reproductive organs, polymorphism etc. were done with material from various museums, but mainly with freshly collected ants from numerous localities in Europe and North America. Several species could be kept in laboratory culture for some time, thus enabling us to study sexual behavior and the relations between the guest ants and their hosts.

Our studies (Francoeur et al., 1985) revealed that all the six guest ant species known and one newly detected by us, share a number of particular behavioral and morphological traits. They all are living as xenobionts with similar relations to their hosts belonging to the genera Formica and Myrmica, respectively. In all species a considerable number of intermorphs, together with ergatomorphs and gynomorphs, is produced. Most, perhaps all, species are functionally monogynous, and gynomorphs as well as intermorphs may be reproductive queens. The $\delta\delta$ represent a regressive evolution from ordinary alate (F. provancheri) to fully wingless and ergatoid shape (F. nitidulus). The number of species and the geographic distribution of the most primitive forms suggest a nearctic origin of the genus.

The genus Formicoxenus, after our revision, now includes 2 species, nitidulus (Nylander) and sibiricus (Forel) (= orientalis Dlussky) from Eurasia, both living with Formica spp., and 5 from North America, chamberlini (Wheeler) (with Manica), diversipilosus (Smith), hirticornis (Emery) (with Formica), provancheri (Emery) and quebecensis Francoeur with Myrmica spp..

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Reduced Egg Laying by workers of the Ant *Leptothorax nylanderi* in Presence of Workers Parasitized by a *Cestoda*

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A worker of the ant *Leptothorax nylanderi* lays more eggs when associated with other ones than isolated, as shown in figure 1. The grouping increases the egg laying by stimulating the vitellogenesis (Plateaux and Saint-Pierre, 1982). *Leptothorax* may be strongly modified by the presence of a parasite *Cestoda* in their body: they are yellow, not layers and solliciters in trophallaxis (Plateaux, 1972).

If two parasitized workers are added to each group of five normal workers, the egg laying of these workers becomes very low, as shown in figure 2. The dissections of workers grouped with parasitized ones are compared with the dissections of grouped workers without parasitized ones. Figure 3 shows the distributions of the two types of workers among four types of ovarian morphology. The difference between the two repartitions is highly significant. This shows that the decrease of egg laying in presence of parasitized workers proceeds partly from a reduction of the vitellogenesis and especially from a retention of the eggs by normal workers. This result may be imputed to a taking of energy made by parasitized workers and to a disturbance of behaviour occasioned by parasitized workers on normal ones.

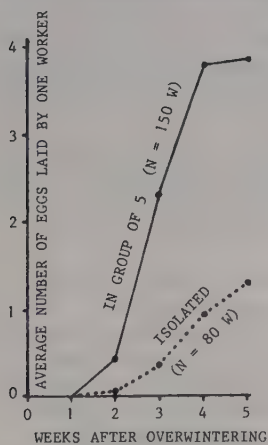


Figure 1: Increase of the egg laying by grouping workers.

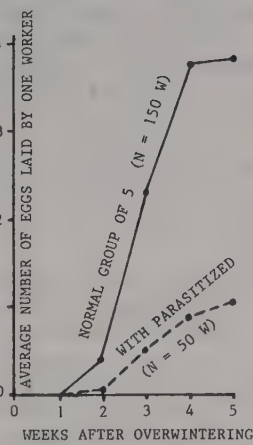


Figure 2: Decrease of the egg laying in presence of parasitized workers.



Figure 3: Ovaries of the workers in group of normal workers (●) and in group with parasitized workers (○). St = Sterile; DV = Diminished Vitellogenesis; RaV = Retention and Vitellogenesis; AL = Active Laying.

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FILM PRESENTATION:

Leptothorax* Ants Parasitized by a *Cestoda**Luc Plateaux* and Laurent Péru***Université Pierre et Marie Curie, Laboratoire d'Évolution, 105 boulevard Raspail, 75006 Paris, France*

The ants *Leptothorax nylanderi* establish their nest in the cavities of pieces of rotten wood which lie on the ground in wet forests. Each colony contains from ten to three hundred workers which are two to three millimeters long. In summer, the nest contains brood, a queen, workers, males and young winged queens. The males are blackish brown, with wings; the queens and workers are reddish yellow with a large blackish brown band on the abdomen.

In some nests, some workers are very different of the other ones, with a golden yellow colour and a smaller average size. These workers have smaller legs, eyes and head, but a bigger petiole. The yellow ants may be queens, but these are rare; intercasts are more frequently observed which correspond to diminished queens and often show a very big petiole. In general, these yellow ants don't lay eggs, their ovaries remain weakly developed, except sometimes in queens.

The behaviour of these yellow ants is indolent: they currently keep on the brood, licking the larvae or doing nothing. They exhibit towards the other workers a petitioner behaviour of solicitors in trophallaxis; they are sometimes solicitors among them, but the meeting of two solicitors don't turn into a lasting trophallaxis.

The yellow ants seem to be rather well admitted in the society, but they sometimes give rise to aggressiveness by the other workers and may be aggressive against each other. It follows that some mutilations of legs and antennae affect these yellow ants.

The yellow ants belong to the same species than the other ones, but they are modified by the presence of a parasite *Cestoda* arrived at the cysticeroid stage. The modifications being realized during the pupation, the *Cestoda* is certainly ingested by the ant during its larval stage (Plateaux, 1972).

The dissection of a yellow worker shows lenticular bodies smaller than an egg (0,25 millimeter) and connected to the gizzard, but floating in the general cavity. These bodies are cysticeroid larvae of the *Cestoda* *Anomotaenia brevis*, each being coated into a cyst; their number varies from one to fourty and more into the same ant. Inside the cysticeroid larvae, a crown of crooks is perceived which are typical of the species; this crown surrounds the extremity of an invaginated rostrum. These crooks will help the implantation of the parasite into its definitive host, for instance a spotted woodpecker, which is known as a host of *Anomotaenia brevis* (Gabrion and al., 1976). When the ant is devoured by a woodpecker, the parasite is released in the alimentary canal of the bird. It contracts violently and comes to devaginate its rostrum and to remove its cyst. It will find a place to fix itself within the papillae of the duodenal bow, by using the crown of crooks and suckers of its rostrum.

The cycle of the Cestoda Anomotaenia brevis may proceed in the laboratory, by using a reared bird with a short development, as a quail (Péru, 1982). New born quails have to ingest some parasitized ants and are raised during three weeks. Then the birds are dissected, their duodenal bow is removed and open from beginning to end.

Anomotaenia brevis is a small Cestoda three to four millimeters long. The last segment (or last "proglottis") is removed and given to eat to a larva of the ant Leptothorax. Incited by a fast of twenty four hours, this larva voraciously devours the proglottis of the Cestoda. This proglottis contains many eggs which hatch into the larva of the ant; they develop into it and become cysticeroid larvae. These larvae finish enlarging during the pupation of the ant, and the larva of Leptothorax becomes, at the end of its development, a yellow adult just like the yellow ants collected in the field. Then the parasite may be stored during a long time, the yellow ants living as long as the normal ones, that is to say two or three years for a worker in the field, and at least the same time for an intercast or an unmated queen.

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FREE COMMUNICATIONS

Recent Developments in Analytical Electrophoretic and Chromatographic Separation Methods for Proteins and Nucleic Acids

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The necessity to gain information from very small quantities of biological material and to detect molecules which are present at very low concentrations has favored the development of micromethods in analytical biochemistry. This is especially the case of electrophoretic and chromatographic methods which have become indispensable tools in the study of the structure and function of biological macromolecules.

Recent developments have been brought to protein and nucleic acid gel electrophoresis. Protein isoelectric focusing as well as two-dimensional separation techniques have also been improved at the level of their resolution and sensitivity. The combined use of ultra-thin gels, of more sensitive stains, of radioactivity amplifiers as well as of protein blotting or immunodetection techniques allows to analyze and visualize proteins amounts lower than one nanogram. For nucleic acids and their fragments such minute quantities can also be identified in some cases.

In the chromatography field, new matrices make high performance liquid chromatography compatible with all types of separations at the analytical and small-scale preparative level (e.g. anion and cation exchange, gel filtration, adsorption, hydrophobic interaction, salting out, reverse phase, mixed-mode and affinity chromatography, and chromatofocusing). Here the lowest amounts which can be separated and monitored on line are in the range of one microgram of protein and one tenth of a microgram of nucleic acid.

Some applications of those new methods to the purification and characterization of proteins, peptides, mono-, oligo-, and polynucleotides, in the field of the study of social insects, will be discussed.

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Did Eusocial Behaviour Arise Only Once in the Apidae?

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Results from a comparative morphological study on the postmentum of bee mouthparts present a new tribal arrangement for the family Apidae and raise the question posed in the title. All non-parasitic members of this family are eusocial except the Euglossinae which are solitary, communal or quasisocial. Traditional classification of the Apidae implies that eusocial behavior evolved at least 3 times in the Apidae (see cladogram below), barring social reversion in the Euglossinae. However, the shape and condition of the postmentum in the Euglossinae suggest that they be placed at the base of the family.

Materials and Terminology

Mouthparts of 101 species from 37 genera and 7 families of bees were examined along with 18 species from 12 genera of Tenthredinidae, Scoliidae and Sphecoidea.

Following Snodgrass (1935) the distal palpus-bearing sclerite of the labium is the prementum and the basal sclerite the postmentum which if divided into separate sclerites then mentum and submentum. The term lorum is used here to refer to a V-shaped submentum.

Results and Discussion

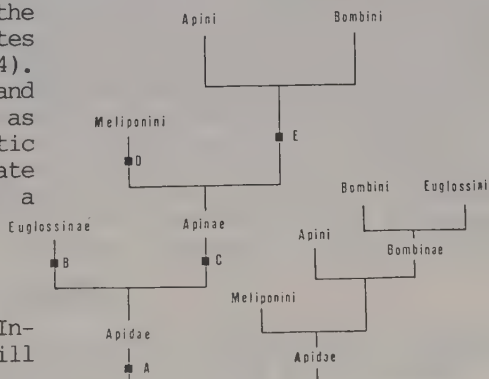
The postmentum of bees has often been mis-identified as two separate sclerites (mentum & submentum). A divided postmentum was only found in the Apidae except the Euglossinae. It is thus considered as a synapomorphy uniting the rest of the Apidae. The plesiomorphic condition, an undivided postmentum, was found in Euglossa and all remaining Apoidea.

It is also considered to belong to the basic mouthpart plan of the Hymenoptera. Much of this deviates from the work of Michener (1984). Since all Meliponini, Apini and Bombini are eusocial and since as proposed they build a monophyletic group, it is parsimonious to state that they probably evolved from a common eusocial ancestor.

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Left: Proposed cladogram for Apidae
Right: Traditional classification

Gene Geographic Studies in *Megabombus pascuorum* Scopoli (Hymenoptera, Apidae)

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A great number of subspecies of *Megabombus pascuorum* are recognized in Europe. Rasmont (1983) has recently regrouped them according to coat colour in a superspecies concept which differs in various respects from earlier groupings of Krüger (1928, 1931).

We have attempted to analyse the genetic relationship of the subspecies on the basis of enzyme electrophoretic data.

Population samples were collected from many European countries but mainly from the zone of contact between Rasmont's semispecies *floralis* and *pascuorum* as well as Krüger's *frey-gessneri*- and *pascuorum*-group (both placed in the semispecies *pascuorum* by Rasmont).

The electrophoretic analysis was based on 20 loci. 19 loci were monomorphic or showed very low levels of polymorphism only. A high degree of polymorphism was observed, however, at the GOT-2 locus, especially in populations of various subspecies of the semispecies *pascuorum*.

This polymorphism involves mainly the two alleles "a" and "b". Allele "a" is fixed in the Corsican subspecies *verhoeffi* and occurs with frequencies of 0.53–0.85 in populations of the central Italian *melleofacies* and the subspecies *pascuorum* in northern Italy. In populations from southern Switzerland it has usually a somewhat lower frequency (0.31–0.75). Allele "a" is also found in populations of the subspecies *intermedius* in northwestern Italy and in its zone of contact with *frey-gessneri* in southeastern France (0.25–0.55), but not in populations of *rufocitrinus* and *maculatus* in the Pyrenees, nor in more western or northern *frey-gessneri* samples from France. Furthermore it was not found in a small sample from Scotland of *septentrionalis*, the northernmost subspecies of the semispecies *pascuorum*.

In the semispecies *floralis* allele "a" is found in higher frequencies (0.17–0.37) only in the zones of contact with Italian populations of the semispecies *pascuorum* in northeastern Italy, southern Switzerland and the north of Yugoslavia, but diminishes rapidly in a clinal fashion towards central Yugoslavia and Hungary. North of the Alps allele "a" has been found in very low frequencies only in some Swiss populations, but not in more northern populations of *floralis* in Germany and Belgium or the Fennoscandian *sparreanus*.

A detailed analysis of the electrophoretic data indicates that gene exchange is restricted not only between the semispecies *floralis* and *pascuorum*, but also between Krüger's "*pascuorum*-" and "*frey-gessneri*-" forms within the semispecies *pascuorum*.

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Biochemical systematics in bumblebees: *Megabombus* s.s. (Hymenoptera, Apidae)

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Delmas (1981) has recently discussed several neighbouring taxa of western European bumblebees, the relative value of which is still debated. He has suggested biochemical and genetic studies to investigate their relationships. With regard to the taxa *Bombus lucorum* and *B. magnus* we have been able to show that a genetic analysis by enzyme electrophoresis is a very useful taxonomical tool (Scholl and Obrecht, 1983; Obrecht and Scholl, 1984). The same methods were now applied to the problematic taxa of *Megabombus* s.s.: *runderatus*/*argillaceus* and *consobrinus*/*gerstaeckeri*. The analysis is based on a sample of twenty loci.

M. ruderatus and *argillaceus* were collected over a wide range of their domains but mainly in their zone of contact in France. *M. consobrinus* was collected in Southern Norway, *M. gerstaeckeri* in the Swiss and Italian Alps. Also included in the analysis were *M. hortorum* (from several European countries), *M. yezoensis* (Japan) and four species of the other European subgenera of *Megabombus*: *M. pascuorum*, *M. pomorum*, *M. mesomelas* and *M. subterraneus*.

M. ruderatus and *M. argillaceus* showed a difference at the GOT-2 locus, where *M. ruderatus* is polymorphic with an allele "a" predominant in French populations and fixed in Italian populations and in *M. r. perezi* (Corsica) and *M. r. sardiniensis* (Sardinia) and an allele "b" which is electrophoretically identical to the GOT-2 allele of *M. argillaceus*. Allele "a" has not been found in *M. argillaceus*, even in areas of sympatry. This indicates that the two taxa do not share a common gene pool.

M. consobrinus and *M. gerstaeckeri* are highly differentiated and do not share alleles at six loci. *M. gerstaeckeri* shows no closer relationship to *M. consobrinus* than to other species of the subgenus *Megabombus*. *M. consobrinus*, however, is closely related to the Japanese species *M. yezoensis* (difference at one locus only).

Cluster analysis (UPGMA-method) of coefficients of genetic identity (\bar{I}) showed that the species of the subgenus *Megabombus* form a compact cluster. The species of the other *Megabombus* subgenera join this cluster at significantly lower levels of genetic identity.

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Allozyme Variation and Differentiation among *Apis* Species

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The honey bee genus *APIS* is generally considered by apidologists to consist of four species. A phylogenetic investigation of the genus was undertaken using data derived from an electrophoretic assay of 18 enzyme loci. In addition to *A. mellifera* from Europe, samples of *A. cerana*, *A. dorsata*, and *A. florea* were obtained from Sri Lanka, where they co-occur within their natural range. A total of 48 colonies were included in the analysis. An initial sequential survey was conducted on starch gels, followed by single condition electrophoresis using the optimal buffer/gel condition for each enzyme (for details see Sheppard and Berlocher, 1985). All four species were run in side-by-side lanes for comparisons of relative mobilities and allozyme congruence in the initial sequential survey. All banding patterns were consistent with a simple genetic basis for the variation.

The distribution of enzyme polymorphisms among the species (and the number of allozymes per polymorphic locus) was as follows: *A. mellifera*- ACON(2), EST(3), MDH(5), ME(3), PGM(2); *A. cerana*- ACON(2), EST(2), MDH(2), ME(2); *A. dorsata*- HEX(2); *A. florea*- ME(2), PGM(3), TPI(2). Seven loci showed fixed allozymic differences among all four honey bee species and three loci were fixed for the same allozyme in all four species. The considerable mean genetic distance among the species (Nei unbiased = 1.30) supports estimates of the antiquity of the genus (20 - 30 mya) made from the fossil record. The amount of genetic change that has accumulated between *A. mellifera* and *A. cerana* (Nei unbiased = 1.10) is almost as extensive as that between *A. dorsata* and *A. florea* ($D = 1.40$), and is surprising in the light of their morphological and behavioral similarities (Ruttner- these proceedings). The debate over the probable phylogeny of the genus is not readily resolved by the data in this study. Based on a simple cladistic analysis, all three possible unrooted trees for the four taxa are supported by the data equally well (or poorly). A UPGMA analysis does support an earlier divergence time for *A. florea* than any of the other species, but fails to resolve the *dorsata*-*cerana*-*mellifera* group.

This study represents the first survey of genetic variation in the genus *Apis*. The extensive electrophoretic differentiation of the species makes it possible to answer a number of questions that still remain concerning the genus, especially the validity of proposed new species.

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Systematic Revision of *Procornitermes* Emerson (Isoptera: Termitidae, Nasutitermitinae)

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INTRODUCTION

Procornitermes, described by Emerson (1952), belongs to the neotropical mandibulate genera group of Nasutitermitinae.

This revision incorporates data from discriminant analysis based on morphometric characters of soldiers and imagos, comparative morphology of mandibles of all known castes, maps of species distribution and collection records on the biology of each species. Elsewhere I redescribed the genus (see note) and the species accepted as valid: *P. araujoi*, *P. striatus*, *P. romani* and *P. lespesii* (= *P. cornutus*).

RESULTS

The discriminant analysis identified necessary and sufficient morphometric characters to discriminate all species, except *P. romani*. This species is known from two soldiers (types). The probability of the types belonging to one of the other 4 groups (species) was calculated as regard to measurements and the results were contradictory. This fact, along with other data discussed elsewhere, indicate that the status of *P. romani* still remains unclear.

Distributional maps revealed that *Procornitermes* species are trans-Andean and extra-Amazonean, occurring in open formations and forested habitats of South America.

The comparative morphology of mandibles of known castes showed that there are intra and interspecific differences between worker and imago, despite this it is possible to state some characteristics for the genus.

The nests of *Procornitermes* consist of earth, saliva and excrements material and may be epigeous (*P. araujoi*) or subterranean (*P. striatus* and *P. lespesii*). *P. lespesii* builds polycalic nests, although the number of "calies" (units) per colony remains unknown. The inner structure looks like an edifice with horizontal levels connected by oblique passages. Each level has a variable number of chambers sustained by pillars which are narrower in the middle. The structure as a whole resembles a winding staircase.

Note: This revision is in press at *Papéis Avulsos de Zoologia*, São Paulo, 36(19)

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1.2 Taxonomy

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Taxonomy of Honeybees

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Honeybees are different from other social insects in many ways, even in taxonomy. This becomes evident when comparing the abundant phylogenetic tree of *Meliponinae* with rich radiation into many genera and subgenera with the scarce ramification in *Apis*. Subfamily *Apinae*, tribe *Apini* and genus *Apis* are all identical in composition, comprising not more than four or perhaps five species. Since at times this modest rank of classification seemed inadequate to the biological importance of honeybees, the taxonomic structure was upgraded by splitting the tribe in several genera and subgenera (*Megapis*, *Micrapis*, *Sigmatapis*; Maa 1953), but this never became popular because a true reason was missing: the small number of highly evolved species remained the same.

THE HONEYBEE SPECIES

At the species level no problems are left for discussion at this time except for two:

1.- Are *A.cerana* and *A.mellifera* two completely isolated species?

There were several reports on transitory types and hybrids in Kashmir. This question can be regarded as solved since it was shown in an investigation together with V.Maul (1983) that after heterospecific instrumental insemination egg fertilization occurs, but that the embryo dies at an early stage. The two species are completely isolated genetically but the process of speciation is not yet advanced to a mature stage.

2.- Does a fifth honeybee species exist?

For a few years Sakagami and collaborators (1980) called attention to the "world's largest honeybee", *Apis laboriosa*. This *dorsata*-related honeybee nests in altitudes of about 3000m on protected places in the southern Himalayan valleys and is of special interest because of its capacity to endure minus temperatures inspite of being of an open-air nesting type. More data on distribution, biology and morphology (male copulatory organs!) will provide a definite answer to the question of the taxonomic rank of this bee.

TAXONOMIC CLASSIFICATION AT THE SUBSPECIES LEVEL

Taxonomic diversification in honeybees is shifted to the lowest rank, the subspecies level. This is probably due to the relatively recent acquisition of new ecological niches of two cavity-nesting species in the temperate zone and in tropical Africa.

It is in this field where the big problems of honeybee taxonomy are found. The first taxonomic attempts in the 19th and up to the middle of this century were made with conventional methods of systematics. That is simply by describing a few museum specimens mainly by color and overall size. Since only quantitative differences are found in subspecies no reasonable classification was achieved.

Modern taxonomy of honeybees started with the introduction of biometrics, later supplemented by multivariate statistics (Alpatov 1929, Goetze 1930, DuPrav 1963). In certain cases a discrimination was achieved even with a small number of characters (Cornuet et al. 1976). To cover the full range of geographic variation many characters have to be used. Out of these, a variable number and arrangement is selected for statistical analysis.

BIOMETRICAL-STATISTICAL ANALYSIS

After years of testing a set of 40 characters was selected for "standard biometry" (Ruttner et al. 1978). Certain rules proved to be of importance:

- 1.- The morphometric characters should include different categories, as size, hair, wing venation and color.
- 2.- Body size can be determined by measuring isolated segments only.
- 3.- Pigmentation shows true geographic variation but it is to be used as one character among many others.
- 4.- To eliminate non-genetic individual variation the means of a sample (in this case 20 bees) should be used.

The results of factor or discriminant analysis are condensed variables which are presented as two or three-dimensional coordinates. Each sample is characterized by the values of the resp. coordinates, each subspecies by the coordinates of the centroids of a cluster. The significance of the established units can be tested by "ellipses of confidence" (Cornuet 1982). A taxon recognized as subspecies should correspond to a level of 75% confidence (Huxley 1939). This provides the morphometric-statistical determination of a taxonomic unit, but a biologist most likely wants, additionally to an abstract formula, a more substantial description in order to visualize the object of his studies. For this purpose selected primary data are given but also secondary values, e.g. proportions which may be significant for certain races: index of slenderness, metatarsal index, cubital index etc.

The following results are derived from the analysis of about 1000 samples of *A.mellifera* and 100 samples of *A.cerana* originating from the major part of the autochthonous areas of distribution.

RESULTS

1.- Graded geographic variability

In general honeybees of higher geographic latitude and higher altitudes are larger, darker and covered with longer hair than those of the tropics and the plains. That is true for *A.cerana* as well as for *A.mellifera*. A geocline was established along the Atlantic coast, extending from Scandinavia to the Cape of Good Hope, thus covering 94° latitude. A similar correlation with altitude was found in East Africa between the coastal honeybee and those as far up as the mountain forest of the Kilimanjaro. This tendency corresponds to various zoogeographic rules in vertebrates, but cannot be generalized for honeybees. The largest and the smallest *mellifera* bee occur in similar latitudes (Morocco and Saudi Arabia). Only slight or no correlations are found between the honeybees of the Black Sea coasts and the Caspian Sea and the Caucasus and Iranian mountains, resp. Variations in biological characteristics which are to be interpreted as ecological adaptations (brood rhythm, reproduction, migration), and therefore correlated with climate, are frequently observed.

2.- Isolation of populations

Mountains, deserts and oceans generally provide complete geographic isolation. But these are not the only isolating mechanisms. Ecological factors are almost as efficient, as is shown in tropical Africa and in the Near East (Ruttner 1984). Permanent hybridization of populations is evidently balanced by continuous two-way selection.

3.- Taxonomic classification

A.- *Apis cerana* F. This taxon was split by MAA (1953) into 11 so-called "species". In a preliminary morphometric analysis only three subspecies were discriminated (Ruttner 1985):

- 1.- *A.c.cerana* F. in all of the mountains and highlands of the north, from Afghanistan to northern China.
- 2.- *A.c.indica* F. in southern Asia. Several island populations are recognizable (Sri Lanka, Sumatra, Java), but with overlapping variations.
- 3.- *A.c.japonica* Radoszkowski on the Japanese Islands.

B.- *Apis mellifera* L.

Many races have been described within this species, frequently without sufficient data. An example will be presented for the methods which can be used for numerical classification of the bees of a given region. The region selected is the northern part of the central Mediterranean (Italy, the Balkans and all of SE Europe from the Alps to the Ukraine), with complex, hard to discriminate bee populations, including two races known everywhere, *ligustica* and *carnica*.

As a first step a principal component analysis was calculated using a total of 252 samples (about 5000 bees) originating from this region. Resulting were different, partly overlapping clusters. These groups were used for a discriminant analysis, which confirmed the classification, showing only a few cases of overlapping (fig.1). Four clusters are very close together (*sicula*, *macedonica*, *carnica*, *cecropia*). Then the homogeneity of the so-called "*carnica*" in the pannonic region between the Alps and the Black Sea was tested. A number of confluent populations was found, but finally only a very simple classification made sense (fig.2): 1.- Bulgaria (B), belonging to *A.m.macedonica*.

2.- *A.m.carnica* composed of two overlapping populations, "*Alpine*" (O) and "*Pannonian*" (R) (including the Carpathians).- Geographic races, of course, have nothing to do with political borders.

The clusters are arranged in the shape of a laying "Y". The stem of the letter is composed of the races of tropical Africa (Ethiopian region), one branch of the Balcan and the Appennine races, the other of West European and North African races. The seven oriental races are found in the area of ramification. For each group of races the number of discriminant characteristics can be given, also for each single race the coordinates of the centroid.

Conclusions

The two species *A.cerana* and *A.mellifera* are split into numerous geographic races. Although in many cases discriminated only by small quantitative morphological differences and fully fertile when hybridized (frequently with strong heterosis effect), the differences in biology may be almost species-like: in South America, European races are being replaced by imported African bees (*A.m.scutellata*) not by hybridization, as should be assumed, but by active extermination (mainly aggressive male competition, migration and increased reproductive swarming). A large array of taxonomic diversification is found at the very periphery of the phylogenetic tree of *Apini*, showing that its ecological potential is not fully exploited. -The frame of existing mor-

phometric data on honeybees provides an insight into the total variability of this species and a sound basis for biological studies.

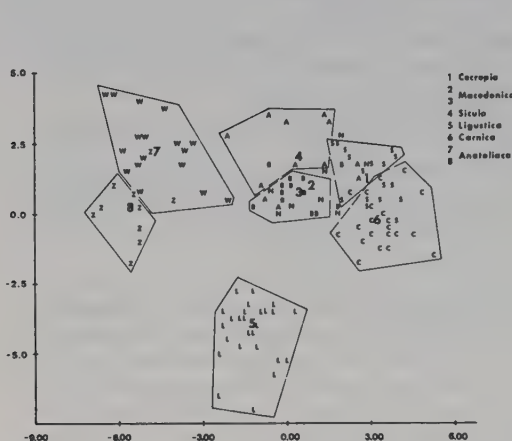


Fig.1.- Clusters found by discriminant analysis of samples from SE Europe and Turkey

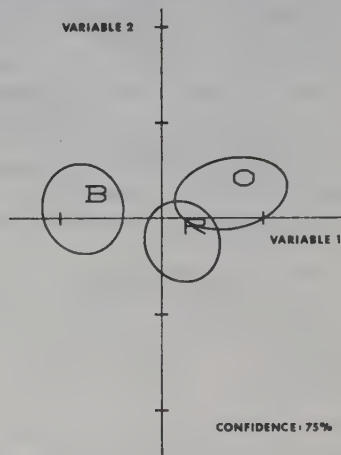


Fig.2.- Ellipses of confidence (75%) of 3 populations of "Carnica". B Bulgaria, O Alpine, R Pannonian

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SYMPOSIUM

Taxonomy and Zoogeography of *Formica rufa* species

Organizers:

Donat Agosti and Cedric A. Collingwood

Taxonomy and Zoogeography of the *Formica rufa* L. Species Group

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Yarrow (1955) revised and keyed the four species found in the British Isles with descriptions based mainly on British examples. Betrem (1960) reviewed the nomenclatural history and taxonomy of this group and added a fifth species from continental Europe. The five species generally recognised by European myrmecologists include F.aquilonia Yarrow, F.lugubris Zetterstedt, F.polyctena Foerster, F.pratensis Retzius and F.rufa Linne.

Extensive collecting over Europe since Betrem (1960) has revealed a considerable complexity of form and variability in the four or five main species, such that the accurate keying and correct taxonomic placement of the twelve or more forms known to present day collectors and ecologists, requires revision and critical re-assessment.

The purpose of this workshop therefore is to review the various forms included in the material presented here so as to arrive at a consensus of opinion as to their taxonomic status and if this is not possible from discussion, to consider and to put into operation as a cooperative exercise, various techniques that could help resolve the placement of problematic forms. Finally the whole problem of speciation in the *F.rufa* species group should be discussed in the context of environmental factors, changing land use, life style and geographical occurrence.

For the purpose of this review the various forms known to occur have been placed into four groups based mainly on their microsculpture as revealed by SEM micrographs of the scutellum and first gaster tergite dorsum of the queen caste. Microsculpture appears to be less variable and to provide better distinctions in some forms than the more generally used chaetotaxy. However this is open to debate and it is intended that other methods of discrimination including enzyme reactions, gland analyses, behavioural characteristics, colony worker size structure and body measurements will be explored during the course of the meeting. Also a clear reappraisal of what can be considered to be a clearly distinct species within this complex group must be a prime consideration in our discussions.

The first group to be examined includes those forms that are closely similar to F.pratensis Retz.. F.pratensis is a generally well known and well documented monogynous, or at most oligogynous monodomous species contrasting with a

polygynous much more local polydomous form described by Goesswald (1951) as F.pratensoides which is probably not a morphologically distinct species but provides a useful example for discussion of monogyny and polygyny in a single species as a possible form of incipient speciation. F.nigricans Emery has closely similar workers and life style to F.pratensis but males and queens have long ciliate hairs on the scale, propodeum and appendages that are absent in F.pratensis but some variability in the development of this character suggests that F.pratensis and F.nigricans may not form entirely separate breeding populations over their large area of overlap in Central Europe. Finally highly polygynous, polydomous populations of F.nigricans are of local occurrence and contrast with the normal monodomous and supposed monogynous situation of the normal form.

The F.aquilonia complex next to be considered provides some real difficulties in interpretation. F.aquilonia itself is a northern boreal species that occurs in some Central European mountain areas. This is the commonest wood ant in northern and central Fennoscandia with large populations occurring in most forest areas. The difficulty here is that there is a tendency for progressive loss of pilosity from north to south in Scandinavia so that individual workers and sometimes whole nest series in South Norway and Central Sweden cannot be safely separated from superficially similar F.polyctena workers while in South-west Finland there is a distinct and quite hairless population that on body size and differences in sculpture differs from surrounding populations of F.aquilonia but on microsculpture is clearly different from continental F.polyctena. Another form somewhat provocatively included in this group is the West Anatolian form of F.rufa as described by Kutter (1975) in which the queens have consistently close gaster micropunctuation similar to that of F.aquilonia although the chaetotaxy but not the microsculpture of all castes is that of F.rufa with some morphological tendency towards F.pratensis. It is a tempting hypothesis to suggest that this approximates to an ancestral form from which the others deviated following the ice retreat northward.

F.lugubris is a generally well characterised species but aberrant forms occur coastally in South Finland and on several Fennoscandian islands including Oland which have some characters comparable with a form from the Alps described by Bondroit (1920) as F.nylanderi. Some of these local populations develop excessive pilosity on the body and appendages including scapes that are outside the range of typical F.lugubris but variability is too great to regard this as a distinct species.

F.rufa itself poses many problems. The monogynous form appears to form a separate breeding system to the polygynous form but has no clear morphological distinction. Some polygynous populations include individuals that could be identified as F.polyctena and there is recent evidence

that the F.rufa complex can be broken down into three overlapping but statistically separable forms based on chaetotaxy (Seifert, in prep.). Workers in some F.rufa series have a few standing occipital hairs and in a few cases queens with hairs on the declivous face of the gaster have been seen, these features creating difficulties in keying. There is a suggestion here that this is a possible sibling species that would be difficult to recognise in all cases.

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Population Genetics of the *Formica rufa* Group

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Five species of the *Formica rufa* group ants are recognized in northern Europe: *F. rufa*, *F. polyctena*, *F. lugubris*, *F. aquilonia* and *F. pratensis*. The taxonomy of the group has appeared problematic because of the existence of (a) morphologically intermediate types, (b) nests with different morphological types present at the same time, and (c) new types which may occur sympatrically or represent geographic differences (Pamilo et al., 1979). These problematic cases could be caused by (1) large intraspecific variation in the morphological characters used, (2) additional sibling species not yet recognized, (3) coexistence of two species in a single nest, or (4) hybridization. The morphologically defined species are known to differ from each other also in respect to the colony types they have (monogyny/polygyny, monodomy/polydomy). The aim of the present study is to examine population genetics of the group in order to understand its taxonomy and the evolution of the colony types. The results reported here are preliminary.

MATERIALS

Material from the five species of the *Formica rufa* group was collected in three localities in southern Finland: Helsinki, Siuntio (40 km west of Helsinki) and Hanko (60 km west of Siuntio). The species were identified on the basis of the hairiness of the head and thorax (Collingwood, 1979, Douwes, 1981). Five to ten individuals from each nest were used for genetic studies. Six polymorphic enzyme genes were examined by means of horizontal starch gel electrophoresis: one esterase (*Est*), one peptidase (*Pep*), malic enzyme (*Me*), glucosephosphate isomerase (*Gpi*), phosphoglucumutase (*Pgm*) and phosphoglycerate kinase (*Pgk*). In total, 19 nests were examined in *F. rufa*, 68 in *F. polyctena*, 12 in *F. lugubris*, 27 in *F. aquilonia* and 24 in *F. pratensis*.

INTERSPECIFIC DIFFERENCES

Formica pratensis is characterized at some loci by specific alleles not detected in any other species, but no diagnostic differences were found between the other four species. The species *F. rufa*, *F. polyctena*, *F. lugubris* and *F. aquilonia* share the same electromorphs in all polymorphic enzymes. However, some frequency differences were observed both between populations of different species and among conspecific populations.

For taxonomic purposes it is best to compare sympatric populations. All four problematic species were sampled at Hanko, within an area of 10 km². The allele (electromorph) frequencies are given in Table 1. *F. lugubris* differs from the other species most clearly at *Pep* but also at *Me*, *Pgm* and *Pgk*. The species *F. rufa*, *F. polyctena* and *F. aquilonia* also differ from each other at several loci. These differences can be seen as differing allele frequencies and as presence/absence of some of

Gene		Gpi			Me			Est			Pgk	
Allele	n	100	67		105	100	85	120	100	80	100	75
rufa	11	.96	.04		.47	.53	-	.05	.78	.17	.94	.06
polychtena	8	.80	.20		.66	.18	.16	.04	.68	.28	.96	.04
lugubris	12	.81	.19		.24	.52	.25	.03	.85	.12	.63	.37
aquilonia	7	.66	.34		.79	.21	-	.34	.66	-	.73	.27

Gene		Pep			Pgm				
Allele		100	80	70	140	120	100	90	70
rufa		.63	.19	.18	.20	.19	.41	.14	.06
polychtena		.95	.05	-	-	.18	.53	.09	.21
lugubris		.18	.76	.07	.05	.01	.82	.10	.02
aquilonia		.95	.05	-	.36	.00	.57	.07	-

Table 1. Allele frequencies in sympatric populations at Hanko; n is the number of nests examined.

the rare alleles. The differences among the sympatric populations indicate that the gene flow between the morphologically distinguished types (species) is - if not completely absent - at least restricted. The fact that nearby nests of different species sometimes share in common one of the rare alleles points to the possibility of hybridization.

INTRASPECIFIC VARIATION

Intraspecific genetic variation was best examined in *F. polychtena* in which populations were sampled in all three geographical areas. Significant differences were detected closely located populations at Siuntio where the species was sampled in three patches of forest, separated by a kilometer from each other (Table 2). This suggests that gene flow can be limited and local populations may genetically differentiate from each other. Further studies on geographic differentiation are required to understand the taxonomy and speciation in the group.

POPULATION STRUCTURES

Population data were also used to estimate genetic relatedness of worker nest mates (Crozier et al., 1984, Pamilo, 1984). Because the Hanko samples were collected from different islands with some isolation by distance, the relatedness values were corrected using equation (15) of Pamilo (1984). The results (Table 3) show that the nests of *F. rufa*, *F. lugubris* and *F. pratensis* are monogynous, or slightly oligogynous, whereas those of *F. polychtena* and *F. aquilonia* are polygynous. This agrees with what is known of the species previously (Pamilo, 1982). The

Table 2. Allele frequencies in *F. polychtena* populations from three different forests at Siuntio; n is the number of nests.

n	Me			Est			Pgk		Pep		Pgm				
	105	100	85	120	100	80	100	75	100	80	140	120	100	90	70
19	.28	.54	.18	.00	1.0	-	1.0	-	.93	.07	-	.10	.78	.12	-
12	.44	.52	.04	.03	.92	.05	.69	.31	.89	.11	.11	.05	.75	.08	-
15	.79	.21	-	.13	.83	.04	.92	.08	.63	.37	-	.02	.68	.27	.03

Species	Population	Nests	Loci	r	SE	F	r*
rufa	Hanko	10	6	.71	.08	.23	.54
polycтена	Siuntio-1	19	3	.06	.05		
	Siuntio-2	12	5	.29	.08		
	Siuntio-3	15	5	.40	.08		
lugubris	Hanko	12	6	.79	.16	.45	.45
aquilonia	Hanko	7	6	.09	.08		
	Helsinki	19	6	.18	.07		
pratensis	Hanko	24	3	.87	.19		

Table 3. Genetic relatedness among worker nest mates; r is the relatedness in reference to the total population, F is the fixation index in the island populations, and r* is the corrected relatedness in reference to the neighbourhood.

genetic data also agree with the studies on colony recognition by R. Rosengren: F.rufa workers reject transfers from other conspecific nests, F.polyctena accepts such transfers when the distance is relatively short (100 m, to the order of magnitude), and F.aquilonia accepts the transfers even when the distance between the nests is 1 km (to the order of magnitude) (Rosengren and Pamilo, 1983). The species also differ from each other in sexual production (proportion of nests producing alates, sex ratio, dispersal of females; Rosengren and Pamilo, 1983, 1986, and the papers of Fortelius and Luther in this volume). The differences in breeding biology and in colony types characterize the morphologically distinguishable species. A more detailed study of the population structures should help us to understand the speciation processes and taxonomy of the group. On the other hand, clarification of taxonomy gives the background required to explain the changes in the population structures. The study continues with the aim to clarify the evolution of the Formica rufa group ants.

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General Discussion

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ROSENGREN introduced the discussion by emphasising five main points for consideration: The increasing patchy distribution and consequent breeding isolation due to alteration of the environment by men; the apparent barrier between monogynous and polygynous populations due to different mating habits; reconsideration of the concept of polytypic species sensu MAYR; the need for an operational taxonomy for well characterised populations which were not easy to distinguish morphologically eg. F.polyctena/aquilonia in South Finland; finally, was the traditional approach of morphological taxonomy a typologist fallacy and was GOESSWALD wrong in his method of distinguishing populations by their life style rather than morphology.

Methods of discrimination that should be considered as a cooperative attempt to sort out the problem include SEM, numerical methods based mainly on chaetotaxy, chromosome studies, light microscopy, behaviour, gel-electrophoreses and chromatography. Individual situations could be confused by mixed nests (Ringrasse).

FRANKE outlined work on chromatographical analyses of mandibular glands and other body parts. There were two trends of characteristic terpenes within the F.rufa group. In comparing the problematic South Finnish F.polyctena/aquilonia with typical Finnish F.aquilonia. Part of the graph was identical between the two populations and part not, suggesting that the two populations are taxonomically close but still distinct. At the same time populations of morphologically identical F.uralensis RUZSKY could differ slightly even from nest to nest in the same area.

SCHMIDT described recent work on isoenzyme studies. Mature queens gave less variable results than young queens but there was too much variability between different castes for sound conclusions and often such tests were inconclusive. Experimental crossing between polygynous forms of the same species and clearly different species was possible but intercross tests had not been carried through to production of f1 generations. Mature queens of different species could be accepted and some mixed populations had resulted but usually with the eclipse of one or the other species in the long term. Mating between different polygynous F.rufa populations was usually successful but had never been observed with the aggressive monogynous form of this species.

DOUWES outlined previous work on chaetotaxy that clearly differentiated four main species: F.aquilonia, F.lugubris, F.polyctena and F.rufa but a small minority of nest series were indeterminate by this method. Hind femur extensor hairs provided the best single character differentiation in most cases. However there were difficult populations between F.rufa and F.polyctena suggesting that these two supposed species were not in fact genetically isolated (E.T.G.ELTON had obtained f1 generation workers from a F.rufaxpolyctena mating). SEIFERT, not present at this meeting, had sent results of a comprehensive study of F.rufa/polyctena based on chaetotaxy showing that this complex could be broken down into three overlapping forms which he proposed to call subspecies i.e. F.rufa ssp. rufa, ssp. intermedia, and ssp. polyctena in defiance of conventional taxonomy where a subspecies must be geographically isolated, but demonstrating the need to recognise such forms nomenclatorically because of ecological and behaviour differences. ROSENGREN again stressed the need for typifying and naming characteristic and stable populations within a species especially with respect to ecological and distribution studies.

ELMES suggested that the whole problem might best be approached by studying populations at their apparent centre of distribution. PISARSKI pointed out that this would not be practicable because of variability within each species from east to west over their whole range and he also drew attention to parallel problems within the F.exsecta NYL. complex. VEPSALAINEN questioned the general aim of the work and wondered whether there should be a reconsideration of the old abandoned ideas of EMERY and FOREL. PAMILO questioned the reality of phenotype invariability.

Finally the discussion was brought to a close through the pressure of time and it was agreed that AGOSTI and COLLINGWOOD should prepare a plan for further cooperative work using well characterised local populations for comparative tests and that fresh thought should be given to formulating a viable species concept. Funding for further work would be required and CHERIX and SCHMIDT suggested that IOBC-West Palearctic Regional Section and the Volkswagen-Stiftung respectively might be approached.

FREE COMMUNICATIONS

A Working Cladogram for the *Vespinae*

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This presentation is part of a long-term taxonomic study of the Vespinae. Phenograms and cladograms based upon the external morphology of the adults have been used to produce a classification. Traditionally the Vespinae are seen to consist of three groups: the nocturnal hornets (*Provespa*), the hornets (*Vespa*) and the wasps (*Vespula*).

The phenogram of the wasps based on 22 characters distinguishes four groups: *Dolichovespula*, *Vespula* s.s., *Rugovespula* and *Paravespula* s.s. with the latter two groups being more closely associated. *Vespula* s.s. is as similar to *Dolichovespula* as to *Paravespula* so that its traditional association with *Paravespula* cannot be maintained. The species-pair *V.squamosa* and *V.sulphurea* are closely similar to and should be included within *Vespula* s.s.

The phenogram of the hornets based on 15 characters distinguishes two major groups plus *V. basalis* and *V. binghami* as an isolated species-pair. Although *V. variabilis* and *V. analis* are associated with the two major groups, their relationships are not clear. The cladogram based on 11 characters maintains the phenogram analysis but in addition assigns *V. variabilis* to one major group and *V. analis* to the other.

The phenogram of the Vespinae based on 19 characters shows *Rugovespula* and *Paravespula* s.s. to be closely associated but widely separated from the rest of the Vespinae. *Vespa* and *Dolichovespula* are also closely associated. *Provespa* and *Vespula* s.s. form two isolated groups but being more closely associated with the *Vespa* + *Dolichovespula* group.

The cladogram of the Vespinae based on 21 characters with *Provespa* as the primitive taxon can be summarised : *Provespa* + (*Vespula* s.s. ((*Dolichovespula* + *Vespa*) + (*Rugovespula* + *Paravespula* s.s.))). *Vespa* is the sister-group of *Dolichovespula* so the traditional taxon *Vespula* is paraphyletic. *Provespa* and *Vespula* s.s. are the primitive groups but each has several unique derived character-states. Compared with the phenogram *Dolichovespula* and *Vespa* are more weakly associated as they share many primitive character-states while *Rugovespula* and *Paravespula* s.s. are still strongly associated as they share many derived character-states.

Following the evolutionary systematic approach of Mayr and Simpson it is proposed the Vespinae consists of five genera : *Provespa* (3), *Vespula* s.s. (10), *Dolichovespula* (18), *Vespa* (23) and *Paravespula* (10), the latter consisting of two subgenera, *Rugovespula* (2) and *Paravespula* s.s. (8). The number of species in each taxon is given in brackets. All other lower groupings will be species-groups.

Taxonomic Studies on the Termite Genus *Odontotermes* (Holmgren) in Kenya

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The genus *Odontotermes* (Holmgren, 1912) belonging to the subfamily Macrotermitinae (fungus-growers) occurs in the Afrotropical region and Asia. It is a large and diverse genus containing some species that are pest in agriculture and forestry. The genus has never been revised and the African fauna remains poorly known taxonomically. Many of the species have similar morphological features and are difficult to separate using classical techniques. Some of the type specimens are only presented by alates while others are of soldiers. 107 species have been described from Africa of which 30 have been described in East Africa. This study has attempted to discriminate *Odontotermes* species using characteristics of morphology and nest structure.

Field collections of *Odontotermes* specimens and the study of nests were made in Kenya, between latitudes 1° and 2°S, and longitudes 36° and 37°E. Detailed external and internal characteristics of the nests and the sculpture of the fungus combs were described. Morphological studies of sterile castes were made and 15 soldier characters were measured from 15 individual termites per nest colony. All available primary types were examined and compared with East African *Odontotermes* material.

Two groups of *Odontotermes* were identified: the 'badius' group and the 'tanganicus' group. They were defined by their differences in head capsule morphology and mandibular features of the soldier caste. In general species in the 'badius' group have wider body and build larger mounds than those in the 'tanganicus' group. Eleven mound types were recognised belonging to ten species identified by their morphometrics and nest structure differences. The Principal Components Analysis confirmed the presence of ten species and showed clear separations of closely related species in both 'badius' and 'tanganicus' groups. Of the ten species distinguished four are described as "sp.near" to certain described species. These are *Odontotermes* sp. nr. *badius*, *O.* sp. nr. *monodon*, *O.* sp. nr. *tanganicus* and *O.* sp. nr. *zambesiensis* and are believed to be new. These findings confirm that very little is known of the African *Odontotermes*. Further investigations involving modern techniques of biochemical characterization will hopefully shed more light on the taxonomy of African Macrotermitinae.

OTU Stability in Phenetics: An Application

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According to Hurd & Moure (1963) the Genus Xylocopa Latr. 1810 comprises 48 subgenera from the New and Old World faunas. Using 42 subgenera represented by males and females, occurring in both faunas, Da Cunha (1983) showed that the Genus Xylocopa is not phenetically homogeneous and that at least two phenae could be recognized.

Since the stability of phenetically produced classificatory schemes may be affected by inclusion of new characters, by addition of OTU's or both, the remaining 6 subgenera are now included in that phenetic analysis, using the same set of characters.

MATERIAL AND METHODS

Males of Ioxylopa and females of Monoxylocopa and Diaxylocopa from the New World fauna plus males of Perixylocopa and Dinoxylocopa and females of Euxylocopa from the Old World fauna were added to the previous analysis. The 45 OTU's were analysed using a total of 106 characters for females and 113 for males. Euclidian Mean Distances were estimated between OTU's using ranged characters and UPGMA cluster analysis performed independently for males and females.

RESULTS

The resulting UPGMA phenograms showed cophenetic correlation $r=0.83$ for males and $r=0.85$ for females.

The included males clustered in the major group proposed by Da Cunha (1983) that shows subgenera from the New and Old Worlds, with Ioxylocopa being equally similar to Aproxycopa and Xylucospilla, the former a subgenera from the Old World. Among females the new OTU's clustered in the same major group and always as part their own faunas. These results indicate a good stability of the phenetics of the unknown OTU's and suggest a systematic study of the Genus.

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Aspects of the Zoogeography of the Ants of Crete (Greece)

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Little attention has been paid recently to the study of the systematics and zoogeography of the ants of Crete, although the biogeographic importance and the high level of endemism of plants and animals of this island are well known. The sources for this zoogeographic study have been the literature and the personal collection of the author.

Forty species of ants have so far been recorded. They are characterised by different types of distribution. Most abundant are those with a mediterranean-s.european type of distribution comprising almost half of the species. This can be contrasted with the distribution types in a mountainous area of northern Greece where mediterranean-central asiatic species dominate. The absence of central european or boreal species is characteristic. Looking at the distribution of ants within Greece, half of the species of Crete are insular or common with southern Greece while half are distributed all over Greece. Of the two endemic species, one is found only on the western part of the island.

Comparing the ants of Crete with those of the nearest regions of Greece, it is seen that they have higher affinities with those of the Cyclades group of islands.

The results of this study agree with the biogeographic trends shown by other groups of plants and animals in Crete such as the totality of plants and the Orthoptera. All these groups show high number of species compared with the area, high endemism and a west to east differentiation in species composition. The high number of species can be explained by the ecological diversity of the island which is due to paleogeographic and human factors. The high endemism and the west to east differentiation must be due to paleogeographic reasons.

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1.3 Morphology and Ultrastructure

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Morphology and Ultrastructure of the Exocrine Glands in Social Hymenoptera

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Social insect communication for the greater part relies on the pheromonal secretions of a wide variety of exocrine glands. Although the majority of these glands are known for a very long time yet, information dealing with their morphological and precise anatomical organization remains restricted to mainly very old contributions from the end of last century.

Only recently, the glandular morphology became more actual again, with most attention being paid to the tegumental abdominal glands in ants (Hölldobler, 1982; Hölldobler and Engel, 1978; Jessen and Maschwitz, 1983) and wasps (Jeanne *et al.*, 1983). Ultrastructural descriptions of the exocrine glands in the social insects are mostly dealing with the termites (Noirot and Quennedey, 1974), whereas the hymenopteran glands so far were only treated in a few specific case studies.

This fragmentary aspect of the ultrastructural reports, and the lack of a general morphological survey of the glands in the social Hymenoptera, have incited us to investigate the fine structure of their glands and start a comparative morphological study in this group.

The number of major exocrine glands per individual varies between 10 to 15, although total numbers around 50 may even be found, when considering the impressive record numbers of 28 abdominal glands in a ponerine ant (Jessen and Maschwitz, 1983), or 16 cephalic glands in vespid wasps (Landolt and Akre, 1979a). In spite of these large numbers, however, all glands belong to one of two clearly distinguishable types according to their cellular organization.

Morphologically most simple are the **glandular epithelia**, in which the epithelial cells secrete directly through the overlying cuticle. In the internally located epithelial glands, such as the formicid post-pharyngeal gland, the labial and Dufour's gland, the secretory product thus reaches a central reservoir space, where it is temporarily stored (Fig. 1A). In the tegumental epithelial glands, as the sternal epithelia in Vespidae and the wax glands of the Apidae, the secretion after cuticular passage reaches the exterior (Fig. 1B), where it eventually may be retained by a well developed tuft of bristle-like setae, as in the wasps (Jeanne *et al.*, 1983), or as wax scales in the bees (Cruz Landim, 1963). A special situation is observed in Pavan's gland, that is only found in dolichoderine and aneuretine ants: the secretory cells form a glandular epithelium underneath the anterior edge of sternite VII, whereas the reservoir is a structurally independent thin-walled sac between sternites VI and VII (Billen, 1985).

The second group glands are composed of numerous **secretory units**, each comprising a secretory cell and a duct cell. An intracellular and

¹ : senior research assistant of the Belgian National Fund for Scientific Research.

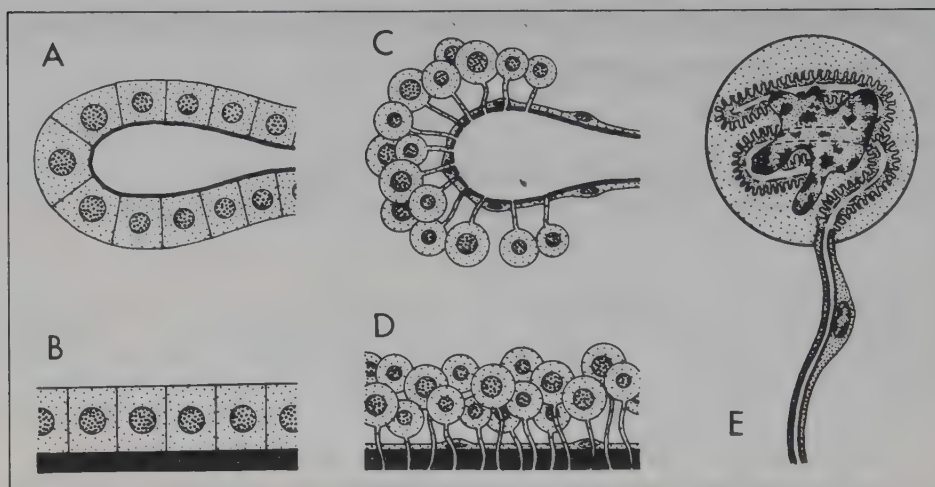


Fig. 1. - Schematic representation of the common exocrine gland types in the social Hymenoptera, in which both the epithelial glands and the secretory unit glands may have a central reservoir (A,C), or directly discharge their secretion through the tegumental cuticle (B,D). The individual unit glands are composed of a secretory cell with an intracellular end apparatus, and a duct cell (E).

cuticular ductule, commonly known as the "end apparatus", collects the secretion from the glandular cell, and continues into the duct cell (Fig. 1E). Also in these secretory unit glands, a reservoir occurs in the internal and intersegmental glands (Fig. 1C), whereas in the segmental glands, such as the wasp sternal glands and the tergal Renner/Baumann glands in the honeybee (Billen *et al.*, 1986), secretion directly reaches the outside (Fig. 1D).

A survey of the hymenopteran glands, as found in the female castes, with respect to these glandular types, is given below. Glands that are only found within one family are indicated with (¹) for the Formicidae, (²) for the Apidae and (³) for the Vespidae. Sternal glands (*) can belong to either of both types. An aberrant situation is found in the labial gland of Vespidae, the acini of which are composed of a large central cell and a number of parietal cells (Landolt and Akre, 1979b). This particular arrangement does not correspond to the normal epithelial type of the labial gland in the other Hymenoptera (□), nor to the type of the secretory unit glands.

epithelial glands	secretory unit glands	
Postpharyngeal gland ¹	Propharyngeal gland ¹	Pygidial gland ¹
Labial gland □	Hypopharyngeal gland	Postpygidial gland ¹
Tibial gland ¹	Mandibular gland	Nasanov's gland ²
Tarsal glands	Mouthpart glands	Renner/Baumann glands ²
Dufour's gland	Metapleural gland ¹	Sternal glands *
Rectal gland	Venom gland	Richard's gland ³
Pavan's gland ¹	Sting valve's gland ¹	Van der Vecht's gland ³
Sternal glands *	Cloacal gland ¹	Intersegmental glands
Wax glands ²	Koschewnikow's gland	

At the ultrastructural level, the secretory cells of both gland types as a rule are characterized by a very extensive smooth endoplasmic reticulum, numerous mitochondria and a mostly well developed Golgi apparatus. The cytoplasmic configuration is in accordance with the elaboration of relatively small, non-proteinaceous molecules, as are the hydrocarbons and lipidic substances that make up the main constituent of the glandular secretions. Exceptions in this regard are the propharyngeal (Formicidae) and hypopharyngeal gland (Apidae), and the venom gland, in which a very well developed rough endoplasmic reticulum occurs. Their corresponding secretions, however, are digestive enzymes and the proteinaceous venom components, respectively. A common feature of all glands are the lamellar inclusions, that probably are to be considered as a stage in the secretory cycle. They can often be observed near to the cuticle, and sometimes are also found in the gland's reservoir.

Because of the ectodermal origin of the glands, their secretory products have to cross a cuticular barrier to reach the reservoir or the outside environment. The microtubular composition of the endocuticle, which is the most prominent part of the cuticle, makes it a permeable layer for the lipidic secretions that are known from most glands. As a result, most epithelial glands display a continuous cuticular intima. Pore canals crossing the cuticle were only found in the wax glands among the bees (Cruz Landim, 1963), the tibial gland of *Crematogaster* and Pavan's gland among the ants (Billen, 1984, 1985), and the abdominal sternal epithelia of the wasps. In the secretory unit glands, the structural organization of the intracellular ductule provides an effective secretory apparatus. The gland cell's contact area surrounding the ductule is considerably enlarged because of its microvillar differentiation, while the cuticular lining of the ductule consists of a permeable endocuticle and a fenestrated epicuticle. The thickened and continuous epicuticular wall of the efferent duct ensures a merely guiding function, and carries the secretion to its storage area prior to final release.

According to their relationship to the cuticle and their secretory pathway, the exocrine gland cells were classified into 3 types by Noirot and Quennedey (1974). Their class 1 cells are in direct contact with the cuticle, and correspond with the epithelial glands, while the class 3 cells with accompanying duct cells represent the secretory unit glands. Class 2 glandular cells have no direct contact with the cuticle, and were only found in the basal region of the sternal glands of termites (Noirot and Quennedey, 1974).

Recent morphological investigations have revealed the existence of exocrine glands that had not been reported before. Because of the modern embedding procedures in plastics, and hence the possibility to section through the hard cuticle, the new discoveries mainly deal with tegumental glands. In this regard, a number of new intersegmental glands in ants were described (Jessen and Maschwitz, 1983), while a review on the occurrence of ant tergal and sternal glands was given by Hölldobler and Engel (1978). The pygidial gland thus was found to be a common structure in all major ant subfamilies (Hölldobler, 1982).

From our own investigations, we recently found the tergal Renner/Baumann glands also in the workers of the honeybee, albeit in a reduced condition. In workers of the cape honeybee, however, they are as well developed as in the queen, thus reflecting the dominant position *capensis* worker bees display when introduced in queenless colonies of other honeybee races (Billen *et al.*, 1986). Another element in the reproductive dominance among the bees are the tarsal glands, the

structure of which was recently described in the honeybee (Lensky *et al.*, 1985). When looking to the tarsal morphology, however, we also found these glands in wasps (Billen, 1986a), ants, and all other, even solitary Hymenoptera we examined so far.

Finally, we revised the morphology of the sting glands in the female Hymenoptera. Noteworthy in this regard is the precise relationship of these glands with the sting itself. The venom gland always opens into the sting base, whereas Dufour's gland only does so in the Formicidae (Billen, 1986b). In the Apidae and Vespidae, on the other hand, the Dufour gland duct closely approaches the sting base, but then bends downwards, and opens into the dorsal vaginal wall. A such fundamental difference undoubtedly will be reflected by the function of these glands. Dufour's gland function in bees and wasps, which so far remains unknown, therefore most probably will be related with their reproductive biology.

Sincere thanks are due to Hilde Zurings for typing of this manuscript.

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FREE COMMUNICATIONS

Changes in Fat Body Ultrastructure During the Fifth Larval Instar in Workers, Queens and Drones of the Honey Bee, *Apis mellifera* L.

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During larval development, the fat body of the honey bee undergoes a series of successive morphological changes, as shown by electron microscopy (Figs. 1-3). This tissue proved to be delicate, and several methods of fixation and staining had to be checked. The cristae of mitochondria (Fig. 3b) could be best visualized by an additional treatment with tannic acid⁺) (Figs. 2 and 3). Using standard methods (Fig. 1), these membranes are not displayed.

The ultrastructural changes are different in the three castes. At the beginning of the fifth instar, growth is predominant. The fat body cells of worker are smaller than those of queen and drone larvae.

Later on, storage phenomena are more conspicuous. Glycogen, however, is found already in younger larvae and increases considerably with the approach of prepupa formation (Figs. 1a, 1b, 2b, 3b).

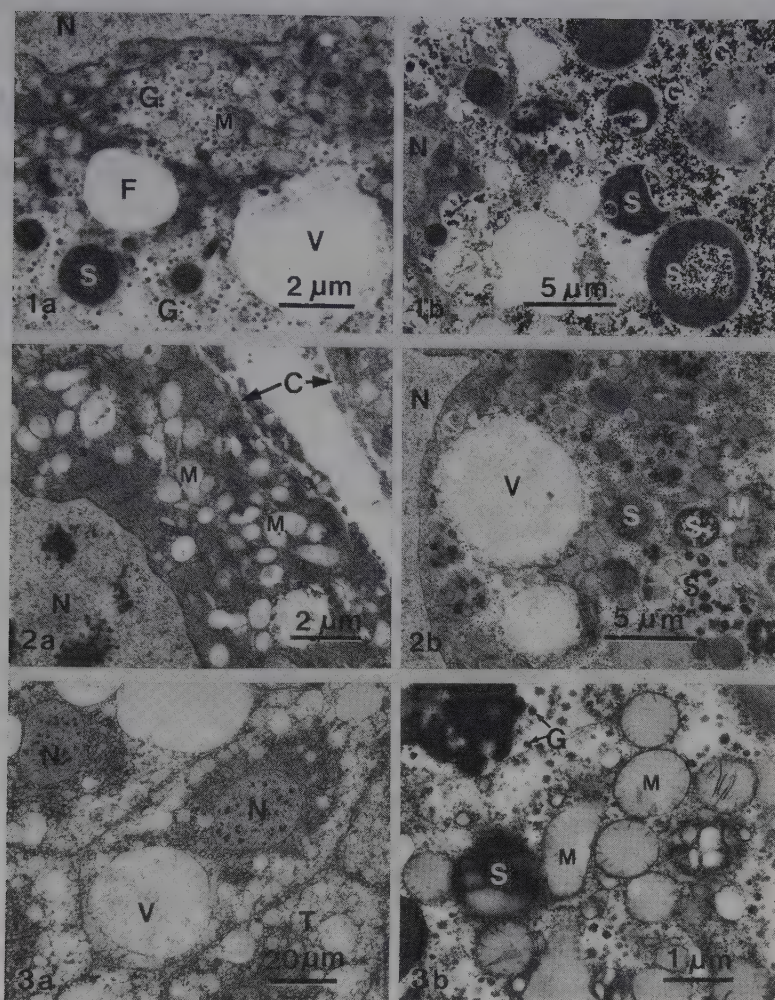
Storage granules (lysosomes), varying in size and shape, begin to appear towards the sealing phase, with an increase in frequency. Two types of storage granules may be observed: first, larger vacuoles containing cell constituents, especially glycogen (Figs. 1a, 3a) which seem to become more condensed during the final developmental phases (Figs. 1b, 2b, 3b); a second type appears later, forming smaller electron dense granules, which increase in size and contain proteins possibly taken up from the haemolymph (Fig. 1a). In the worker, the granules are often ring-like (Fig. 1b).

Fat droplets and larger vacuoles with/without inclusions of cytoplasmic material (see above) are also present. Finally, the fat body disintegrates, and the cells reveal signs of autolysis.

These changes occur earlier in development and to a higher degree in the worker (Fig. 1a and b), whereas the structures in the queen seem to be well preserved over a longer period before pupation (Fig. 2a and b); the drone occupy a medium range (Fig. 3a and b).

The mitochondria show a high variability in size and shape as well as in number of cristae.

⁺) Technique: The treatment with tannic acid (1% in cacodylate buffer, 1 hr at room temperature) has been applied following prefixation with glutaraldehyde (2.5% in cacodylate buffer, 0.1 M, pH 7.3, 6 hrs at 4°C), and prior to postfixation with osmium tetroxide.



Fat body of the honey bee, *Apis mellifera*, 5th larval instar

Fig. 1. - WORKER. a) Before sealing, Magnification 6 000 x, b) Prepupa, 3 000 x. Fig. 2. - QUEEN. a) Early 5th instar, 6 000 x; b) Prepupa, 3 000 x. Fig. 3. - DRONE. a) Early 5th instar, 440 x; b) Before prepupa formation, 11 000 x.

C cell surface, F fat droplet, G glycogen, M mitochondrion, N nucleus, S storage granule.

Compounds of Honeybee's Peritrophic Membrane

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In honeybee imagos (*Apis mellifica* L.) the peritrophic membrane is generated not from single specialized cells in the anterior region but in the whole midgut (Hering, 1939).

MATERIAL AND METHODS

In two stages of pupae (PD and PDM, see Rembold et al., 1980), in just hatched, 1, 3 and 8 day old bees, in foragers and in winter bees, compounds and formation of peritrophic membranes were investigated. Midguts were dissected out and after fixation with Carnoy or formaldehyde the following stains were used: PAS, BAUER, HALE-PAS, metachromatic staining with TOLUIDINE-BLUE partly after treatment with hyaluronidase, ALCIAN BLUE at different pH and with different concentrations of $MgCl_2$, SUDAN BLACK B, NINHYDRIN, and MILLON.

RESULTS

In pupae a secretion of substances can be seen that can be found later also in the peritrophic membrane of imagos. Even hatching bees have a peritrophic membrane that does not differ much in its compounds from older ones. In all imago stages the PM consists of membranes and granules and contains proteins, neutral and acid mucopolysaccharides. Acid mucopolysaccharides are scarcely or not sulfated, the acid group seems to be carboxylic. Less hyaluronic acid is present in all imago stages. Young bees have more acid and less neutral mucopolysaccharides than foragers and winter bees. Only in winter bees are there traces of lipids in the peritrophic membrane. Further studies on the localisation of chitin in the peritrophic membrane will follow.

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Light and Electron Microscopic Study of Oenocytes in Adult Honey Bees with Particular Emphasis on the Fixative Osmolarity

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INTRODUCTION

The oenocyte development in honeybees has been studied by Boehm (1965) and Kramer (1962). On paraffin sections prepared as described before (Raes et al., 1985), oenocytes showed cytological changes which have been interpreted as signs of cellular activity. However, they occur in all bees studied, regardless of the season, or their age. The fact that these changes could not be found on resin sections made us suspect that they might be fixation artefacts due to the uncontrolled fixative osmolarity, customary in light microscopic histology. We therefore studied the effect of this parameter on oenocyte cytology, quantitatively and qualitatively, at the light and electron microscopic level.

MATERIAL AND METHODS

Per situation 150 crosssections were drawn at the light microscopic level; the drawings were measured by automatic image analysis (IBAS II; KONTRON). For T.E.M. the tissue was prepared as described (Raes et al., in preparation). To 0.1 M cacodylate buffer 0.0, 0.07, 0.12, 0.18 and 0.23 M sucrose were added (vehicle osmolarities of 235, 310, 365, 430 and 485 mOSM respectively).

RESULTS

The two lowest osmolarities cause a significant cellular swelling, and cytological changes comparable to those mentioned before. At the E.M. level we found mitochondrial swelling, vesiculation of the S.E.R. and vacuole formation. As the highest osmolarities provoked some cellular shrinking, which obscured membrane contrast, a vehicle osmolarity of 365 was chosen for further experiments. Under this condition, the crosssection measurements of oenocytes show a regular, steep rise from the first till the 10th day of adult life. In this period of cellular growth, which is mainly due to a considerable increase of the S.E.R., cell wall invaginations containing more or less electron dense material come close to the nucleus. After the 10th day, the cellular volume decreases steadily; large scale autophagocytosis, resulting in an increasing amount of lipofuscin accumulations are typical for this period. Lipid vacuoles can now regularly be seen in the cytoplasm where the S.E.R. becomes gradually more compact and therefore more electron dense. A systematic T.E.M. description of this cell cycle is in preparation. Our tentative results support the idea that oenocytes have a function in relation to wax synthesis.

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Morphology and Ultrastructure of the Thoracic Salivary Gland of the Worker Honeybee

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The most detailed description of the morphology and histology of the thoracic salivary gland of Apis mellifica is still that by Schiemenz from 1883. For more recent references see Cruz-Landim (1967, 1968). This gland was not yet investigated electron microscopically in Apis, but in a species of Bombus (Agren, 1975), Melipona, Xylocopa, Megachile, and Colletes (Cruz-Landim, 1968).

The glands are paired, each half consisting of two parts, a smaller anterior or 'external', and a larger medial or 'internal lobe'. These correspond to the description of Kapil (1958) for A. cerana, but are not mentioned in most descriptions for A. mellifera (e.g. Snodgrass, 1956). Each lobe consists of densely packed tubulous acini, typically with a diameter of 30 - 40 μ m. Frequently, pieces of 0.5 mm and longer, were seen, which is much longer than in Bombus (Agren, 1975). In contrast to previous studies (Cruz-Landim, 1967), dichotomous or multiple ramifications are frequent, on average 1.5 ramifications per 1 mm of acinous tubuli. Less frequent than the acini are ducts, with a diameter of ca. 20 - 25 μ m. Anterior-medially there is a thin-walled reservoir packed within the acini, the cell bodies of which protrude into its lumen and form taenidia.

The acini are composed of two types of cells, corresponding to type I and II from Agren (1975). The ducts are composed of cells similar to type IV; type III cells were not found. In our material, the secretory cells (type I) contain almost no Golgi complexes, but abundant rough endoplasmic reticulum, which is characteristically vesicular. Their nuclei frequently contain fibrous bundles.

In contrast to Agren (1975) and Cruz-Landim (1968) we found some profiles, adjacent to the acini, which are interpreted as axonal. These contain dark, presumably neurosecretory, vesicles.

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Iron-Containing Cells in the Stingless Bee *Scaptotrigona postica* Latreille (Hymenoptera, Apidae)

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The orientation of a variety of animals is affected by magnetic fields. After the discovery of the magnetotactic bacteria (Blakemore, 1975) with their magnetite particles - the magnetosomes; iron-rich cells were found in the honey bee (Kuterbach *et al.*, 1982). Now, we report the presence of some particles that structurally resemble bacterial magnetosomes and their site of synthesis in the stingless bee *Scaptotrigona postica*.

MATERIAL AND METHODS

For light microscopy, larva, pupa and adults of *S.postica* were fixed and then embedded in plastic. Sections (3 μ m thick) were stained for iron with the acidic potassium ferrocyanide. For electron microscopy, the fat tissue of foraging bees was prepared conventionally and embedded in Spurr. Silver sections were stained with uranyl acetate and lead citrate.

RESULTS

The blue iron granules appeared in the periphery or attached to the nuclear membrane of the epithelial cells of the larvae ventriculus, and in the trophocytes found mostly in the abdomen of the adult bees. Positive staining was also found in the epithelial cells of the mid-gut and in some pollen granules present in the mid-gut lumen of nurse bees, and in the Malpighian tubules cells of old foraging bees. Ultrastructurally the iron particles are membrane bounded and they appeared inside or in close relation to the rough endoplasmic reticulum and in the perinuclear space. Some round particles, ranging from 400 to 1600 Å in size, structurally resemble bacterial magnetosomes. As suggested by LM and EM data, probably the magnetosomes are synthesized at nuclear envelope of fat cells level with iron absorbed in the mid-gut from some kind of pollen granules.

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Ultrastructure, Spatial Distribution, and Frequency of Antennal Sensilla in the African Termite *Schedorhinotermes lamanianus*

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Termite workers and soldiers lack any eyes. Thus the complex social organisation in termite colonies has to be regulated by mechanical and mainly by chemical signals. Olfactory signals are predominantly sensed by antennal sensilla. The hair-sensilla on the antennal flagellum of *Schedorhinotermes lamanianus* were investigated with the scanning- and the transmission-electron microscope. The numbers both of sensilla and of sensory cells are larger in workers (1900/5000) than in minor (1600/4670) or in major soldiers (1550/4600). Workers, minor soldiers, and major soldiers show a distinct polyethism within the colony.

Different types of sensilla were distinguished on the basis of their external shape and on physiologically relevant criteria such as the wall structure, the number of sensory cells, and the specialisation of their outer dendritic segments. In general the types describes here show similarities with those in cockroaches (SCHALLER, L. 1982: Cell Tissue Res. 225, 129 - 142 and quotations therein).

Grooved-surface sensilla (at least 3 different types) constantly contain one dendrite ending with a tubular body at the base of the hair - which is typical for mechanoreceptors. The other 1 to 5 dendrites enter unbranched into one of the two hair lumina and, presumably, run to the terminal porus. 6 Type-I sensilla and 7 Type-II sensilla occur in a symmetrical pattern on each flagellar segment. Type-III/VI sensilla represent about 15 % of the sensillum population on the distal part of the flagellum. Sensilla with a tubular bodies and terminal porus are multimodal: mechanosensitive and contact-chemosensitive.

A single no-pore sensillum is always located in a ventral position on each segment of the distal half of the flagellum. The unbranched dendrites are tightly enclosed by the wall of the peg which lacks any pores. They might be thermo-hygroresponsive.

Single-walled sensilla are characterized by pore tubules penetrating the hair cuticle and by branched outer dendritic segments. They can be separated into two types: Type SW-I sensilla are very rare constituting only <3% of the sensillum population. They are located only at the central part of the antennal segments. The second type (SW-X) is a morphologically inhomogeneous group of sensilla with variable external length and shape. They have a conspicuous variation in the number of sensory cells - either two or four. The number of SW-X sensilla is higher in termite workers (110 sensilla) than in minor soldiers (90 sensilla).

Double-walled sensilla are short, blunt pegs. Their hair shaft is externally grooved. The hair walls are pierced with spoke channels leading from the longitudinal groove channels to the inner receptorlymph. This type shows three unbranched dendrites within the hair shaft. It occurs more often in minor soldiers (23%) than in workers (18%). Double-walled sensilla are asymmetrically distributed on the flagellar segments with a concentration on the ventral-medial side of the antenna.

Workers and minor soldiers show a polyethism in the colony, mainly during foraging. It is likely that the caste-specific variation and the frequency of sensilla and the asymmetric distribution of the Type-DW sensilla is behaviourally important.

Histological and Anatomical Observations on the Ovary of the Parasitic Ant *Sifolinia lemasnei* Bernard

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The number of ovarioles per ovary is highly variable in insects, ranging from one to as many as thousands. Even within a single species, for example *Formica lugubris*, a polygynous species, queens exhibit between 120 and 224 ovarioles and workers between 2 and 14 (Loosli & Wüest, 1982). We had the occasion of studying the rather rare parasitic species *Sifolinia lemasnei* Bernard and we thought interesting to check ovariole number and nurse cell number, which is generally estimated to be 15 in Formicidae (Grassé, 1951).

MATERIAL AND METHODS

Females of the parasitic ant *Sifolinia lemasnei* Bernard (collected or reared from a nest of *Myrmica sabuleti* from Spain by X. Espadaler, to whom I express my best thanks for that supply) were studied morphologically and histologically at the level of their ovaries. Serial slices were drawn in camera lucida and reconstructions of ovaries and oocyte chambers were done.

RESULTS

The reconstructed schemes allowed us to follow the ovariole tubes, which were found to be 3 or 4 per ovary. In our preparations, sperm seems to be stored in a simple folding in the roof of the bursa copulatrix; sperm remains concentrated in a single mass, exactly at the same place in freshly hatched and egg laying females. This must be ascertained by further studies and puts the question of how fecundation takes place or not (for male eggs).

For the oocyte chambers, reconstructed schemes allowed us to estimate the number of nurse cells to about 16 - 17, although the theoretical number should be 15.



Ovary



Oocyte chamber

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1.4.1 Polyethism and Polymorphism

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Ethogenesis of the Social Insect

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Until lately, the behavioural evolution of an individual social insect during its life was considered to be merely a consequence of :

- its maturation-linked to age,
- its size and- or its shape,
- the "needs" of the society, as expressed by the social regulations.

Therefore, we can say that ethogenesis (that part of individual's ontogeny which relates specifically to behaviour) was considered mainly as an endogenous process, developing in response to- and modulated by- social pressures. This "classical" point of view did not prevent research into more complex mechanisms from taking place. In particular, one of these mechanisms is early individual learning, recently emphasised by the consequences of the kinship theory.

Most of the recent computer-based studies have confirmed the pioneering results, particularly those of the German researchers on the ethological development of the individual social insect (e.g. works from Gosswald's and Lindauer's schools respectively on the red wood ant and the honey bee). Usually, the young individual starts his imaginal life by a short, inactive period preceding the appearance of nursing behaviour. Later, the individual is more devoted to domestic tasks before developing the last phase of his social life, marked by foraging activities.

These general rules of individual ethogenesis can be accelerated or slowed down in relation with the ontogeny of the society. For instance, the recording of the behavioural acts performed by individually marked ants of Ectatomma ruidum (Ponerinae) shows that the period of inactivity is considerably shortened in newly founded nests until there are about 7 workers ; after which, the period becomes comparable to that in a society of 80 adult workers (Lachaud and Fresneau, 1986).

The social stimuli received during a short period following the imaginal hatching can also modulate or alter the ethogenesis. For instance, in the red wood ant Formica polyctena Först., the absence of cocoons during the first week after hatching considerably decreases the nursing behaviour when following the callow maturation (Jaisson and Fresneau, 1978). On the other hand, when a queen is present during that early period, the workers develop a more active tending behaviour pattern (Jaisson, 1975a). The queen has probably a non-specific effect on workers by stimulating the nursing behaviour. In the ponerine ant Ectatomma tuberculatum Roger, workers individually isolated at 2 days old for ten days show a stable but low level of nursing behaviour and a

high level of inactivity after reintroduction in their society until at least forty five days old (Champalbert and Jaisson, 1985 ; Champalbert, 1986).

Most studies carried out on ethogenesis of the social insect relate to the problem of stimulus recognition. It has been demonstrated that imprinting-like phenomena are often involved in the individual showing a more or less high level of flexibility and adaptability. The kind of stimulus the social insect is able to recognise after early learning can concern the milieu (e.g. recognition of plant odours) or other individuals (species-specific recognition or kin recognition). Recognition between closely related individuals is indispensable for the validity of the kin selection theory. It is based on the perception of chemical components studied in detail elsewhere in this volume. The ethological aspect of the problem has been explored primarily at the species-specific level. The model of mixed interspecific societies has been used in ants since Fielde (1903 ; see review by Jaisson, 1985). This model is not totally artificial because various examples are known of mixed colonies in the natural environment. It is the case, particularly, for dulotic ants, whose evolutionary originality has been to turn to their profit inter-specifically a phenomenon that normally takes place intra-specifically. Authors have demonstrated that, in fact, the enslaved species are able to learn how to recognise the adults and brood of the dulotic one (Jaisson, 1975b ; Le Moli and Mori, 1986). This is, doubtlessly, linked to the phylogenetic proximity of the parasite and the host. In this way, Errard and Jaisson (1984) have demonstrated that age and phylogenetic distance negatively affect the feasibility of an artificial mixed colony. We believe that species-specific recognition and kin recognition belong fundamentally to the same ethological process during ethogenesis.

Several results have suggested that the construction of the recognition of the colonial chemical visa starts very early in the individual's life. In two *Polistes* species, for instance, Pfennig et al. (1983) have characterised a 1 to 2 hours sensitive period following the post-imaginal hatching. In the ant *Camponotus vagus* Scop., Morel (1983) showed that the fact of having made the imaginal moult within the colony is important for the ethogenesis of nestmate recognition. Moreover, Isingrini et al. (1985) recently demonstrated, in the ant *Cataglyphis cursor* Fonscolombe, that the chemical visa of the colony is learnt during the larval stage. As a consequence kin recognition, as measured by preferential nursing behaviour (licking and carrying), takes place very early in the worker's life and does not seem to depend on an endogenous, innate program.

Unfortunately, the litterature on kin recognition does not emphasise the importance of the ethological process by which kin recognition takes place and seems to be satisfied by the trivial relationship between genotype and phenotype. Conversely, we believe that the knowledge of the ethological way by which group selection based on kin has evolved in social insects is important. Many studies show that the relationship between kinship and altruistic behaviour is not necessarily direct. The spatial proximity between individuals, coupled with behavioural plasticity or conditionability during ethogenesis can lead to the establishment of what we shall call fellowship. Fellowship can be defined as the relatedness between individuals

directly based on recognition (between kin or non-kin). Fellowship should be the necessary ethological step between kinship and altruism. This ethological process has been selected for during evolution as part of a co- development of insect societies and the particular form of kinship selection they represent. We think that this model enriches the classical kinship theory by taking into account the complex ethological mechanisms involved. These mechanisms may both benefit closely related individuals (kin selection) or genetically distant individuals (dulotism, robbery).

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SYMPOSIUM

Polyethism - Polymorphism and Integrative Behaviour

Organizer: Reinhard H. Leuthold

Introduction

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"Integrative behaviour" is a key-word in the study of social insects. An insect society is in principle the sum of its individuals. However, it is more than this. With the development of the collective body, the single individuals adopt new repertoires of behaviour adapted to the higher colonial level. The colony can become an integrated behavioural or even physical entity that functions as a superindividual organism, as is clearly demonstrated by a termite mound.

Division of labour is the most important step towards social behaviour, reproductive division of labour is the most basic one. With a higher degree of social organization we usually find increased specialization of the individuals. The efficiency of the colony may be improved at the cost of individual identity. With a higher degree of evolution, behavioural specialization is reinforced by physical caste polymorphism. The aim of this symposium is to study behavioural integration. Polyethism and polymorphism are the visible characteristics to be analysed. The first series of papers are descriptive and provide basic information for all further biological questions; the next series deals with mechanisms controlling social organization, and the third reveals principles of integrative behaviour, some of which are mainly centered around theoretical questions.

DESCRIPTIVE ANALYSES

1. The physical result of social organization becomes apparent in the colony structure. R.W. Klein (poster=P) reports on different constitutions in closely related twig-dwelling, primitive ants, Pseudomyrmex: monogyny, polydomy - polygyny, monodomy, or oligodomy, an excellent object for comparative sociobehavioural studies.
2. The basis of social organization may be a kinship or an assembly of individuals, i.e. a group. The latter case is described by R.E. Conconi et al. (P) for ant settler queens. It is interesting to note that a tendency towards polyethism is found in such groupings.
3. K. Tsuji (P) reports on queenless Myrmicine ant societies. Even in the absence of a queen, nestmates are recognized and polyethic organization is well developed.
4. In contrast, C. Brandao (lecture=L) shows that in another Myrmicine ant it is the influence of the queen that gives rise to a high level of social polyethism.
5. and 6. Among primitive ants (Ponerinae) A. Dejean et al. (2P) demonstrate clear patterns of polymorphism and age polyethism.
7. The principles of age and caste polyethism, as far as they are known in termites, are summarized and updated by E. McMahan (P).
8. New findings concerning a complex organization and division of labour between minor and major workers during the process of food acquisition in Macrotermes are described by J.A. Lys and R. Leuthold (L). These are the first records on extranidal, polyethic organization in termites.

9. and 10. Extranidal division of labour in Formica colonies reveals evidence for a highly sophisticated optimization between territorial defence and food acquisition, as reported by R. Rosengren (L). L. Sundström (P) describes the distribution of tasks between workers of different sizes and demonstrates a relationship between this distribution and the distance covered away from the nest.

MECHANISMS CONTROLLING SOCIAL ORGANIZATION

11. Daylength may be a key factor for the change of seasonal polyethism in honey bees. P. Fluri and S. Bogdanov (P) were able to demonstrate physiological changes in worker bees depending on photo-period.
12. Individuals in ant colonies are active in coordinated, rhythmic, colony-generated cycles of 15 to 36 min. With this finding, N. Franks and S. Bryant (L) give splendid evidence for a colonial mechanism that coordinates individual behavioural interactions.
13. Age polyethism is mediated by JH titer in ageing honey bees. G. Robinson's model (L) predicts a JH-dependent response threshold of task-associated stimuli.
14. Whereas in most insect societies communication is a key factor for social integrative behaviour, J.L. Deneubourg et al. present a model for foraging regulation in ponerine ants based on individual success without communication (see Symposium "Foraging strategies").

PRINCIPLES OF INTEGRATIVE BEHAVIOUR

How can a social colony regulate the division of tasks in order to become an optimized functional body?

15. and 16. D. Fresneau et al. (L) and B. Corbara et al. (P) show that individual characteristics (idiosyncrasy), combined with more flexible individuals, give rise to homeostatic regulation of task distribution in ponerine ant colonies.
17. A similar principle was found by M.J. Sommeijer (L) in stingless bees. He recognizes the role of specialists and generalists and discusses the adaptive significance on an individual versus colonial level.
18. Polyethic flexibility has been demonstrated in a dimorphic Pheidole ant species by P. Calabi and J. Traniello (L). It is considered to be a mechanism which quickly adapts the colony to its changing needs: an adaptive alternative to flexibility in caste ratio formation.
19. S. Beshers and F. Traniello (P) found polyethic specialization through learning by pressure of colony needs in higher myrmicine ants.
20. K. Jaffe (L), on the other hand, reports on the tendency among Attni ant species, that high social organization is attained at the cost of individual learning capacity. This is an interesting aspect in relation to questions of evolution.
21. An insect colony is a superindividual entity. This initially accepted view is fully expressed by D. Gordon (P), who found in harvester ants that the efficiency of individual task specialization increased with ontogenetic change in colony organization, and not through accumulated experience of individual insects. A colony as an integrated organism of a higher order develops ontogenetically. This is one of the comprehensive conclusions of the symposium.

Colony Structures of Three Species of *Pseudomyrmex* (Hymenoptera: Formicidae: Pseudomyrmecinae) in Florida

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Ants that live in small polydomous colonies may be profitable objects for comparative investigations on the evolution and ecological significance of polygyny, social parasitism, or worker reproduction (e.g., Alloway et al., 1982). In this light, the twig inhabiting ants of the genus *Pseudomyrmex* may deserve more attention than previously granted. This paper takes a first step in this direction by summarizing data on the colony structures of three related species of *Pseudomyrmex* common in Florida: *P. ejectus* (F. Smith), *P. pallidus* (F. Smith) and *P. seminole* Ward. These species, to which Ward (1985) gives a key, often co-occur in open field habitats.

MATERIALS AND METHODS

The data are based on dissections of 77 nests (twigs or weed stems inhabited by the ants) of *P. ejectus*, 46 of *P. pallidus*, and 89 of *P. seminole*, collected in the vicinities of Gainesville, North-Central, and Homestead, South Florida, between Oct. 81 and Aug. 85.

RESULTS

All species are polydomous, with following numbers of queenless nests for each queenright nest: *P. ejectus*: 1.3; *P. pallidus*: 0.9; *P. seminole*: 8.9. Average (in parentheses: maximum) worker populations of queenright nests were larger than those of queen less nests: *P. ejectus*: 45.9 (149) vs. 26.3 (70); *P. pallidus*: 31.0 (107) vs. 15.0 (40); *P. seminole*: 93.0 (206) vs. 23.6 (79). In all species, immatures were present in large numbers throughout the year, averaging ca. 80% of the total population. Polygynous nests occurred in *P. pallidus* (32.6% of all nests; up to 23 queens) and *P. ejectus* (15.6% of all nests; up to 10 queens), but not in *P. seminole*. Nests of *P. pallidus* from the same collecting sites fused easily in the laboratory, exhibiting only weak (if any) mutual aggression. *P. ejectus* and *P. seminole*, in contrast, showed pronounced intercolonial aggression. In all species, queenless nests accounted for the bulk of alate production: *P. ejectus*: 88.1% of all alates (adults and pupae) were in queenless nests vs. only 44.5% of all workers; *P. pallidus*: 70.6% vs. 27.6%; *P. seminole*: 94.4% vs. 70%. Sex investment ratios were male biased in *P. pallidus* (1.2:1) as expected for a polygynous species (Trivers and Hare, 1976), but female biased in *P. ejectus* (1:2.35) and *P. seminole* (1:1.64). In contrast to *P. ejectus* and *P. seminole*,

queens of P. pallidus laboratory colonies often mated on or near their nest tubes and sometimes were permanently readmitted to their mother colonies. Interestingly, P. leptosus Ward, a workerless inquiline (Klein, 1986), is closely related to P. pallidus.

(Research conducted at: University of Florida, Gainesville)

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Polyethism of Settler Queens of *Liometopum apiculatum* M. and *Liometopum occidentale* Var. *Luctuosum* W. (Hymenoptera-Formicidae-Tapinomini)

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INTRODUCTION:

"Escamoles" are among the most searched edible insects because of their very good flavor (Conconi, J. R. E., et al 1984a, b). This year they cost \$60 U.S. Dollars/Kilo. They belongs to the immature stages of the reproductive cast of the *Liometopum* genus ants. Nothing was known before about the behaviour of settler queens of these ant genus, being interesting to compare *L. apiculatum* whose colony foundation is made by only one queen, and *L. occidentale* made by 3 to 40 queens.

MATERIAL AND METHODS:

Queens of both species were marked using microlabels having each one a different number of sign. Several photographs were taken with a Minolta camera each half and hour during 24-83 hours in six foundations of *L. apiculatum* and in 1,3,9,10 queens in *L. occidentale*. Each exposition was analyzed under a stereoscopic microscope in order to know their behavioural repertory. Data were analyzed in a Columbia computer with an energraphics program.

RESULTS:

In *L. apiculatum*, we found that behaviour pattern was not uniform among the studied queens. Nevertheless, the rates of exploring, moving out nest excavating and toilettes were usually found higher through the light period day, on the opposite, the oviposition degree, brood care, remain over the brood and inactivity increased during the night. In foundations constituted by 9 or 10 queens of *L. occidentale* var. *luctuosum*, we found that the major activity was observed at night. Besides, it exist a labor division among settler queens although it is not divided in a proportional or clear form because the lapses dedicated to some labor are variable. Some queens are more active than others and work harder, also some are more specialized than others in one function, by example, one or two queens dedicated more time to the brood care, others to patrol the nest area, etc. The activity rates of each queen behaviour pattern during 24 hours did not persist but they changed as the time went (83 hs.). If settler queens are only three, the brood care still keeping the main activity, but they remain more time over the brood. Oviposition degree and the exploring time increased. The trophallaxia and patrol activity decreased. Also inactivity decreases greatly until it disappeared completely in one queen foundation.

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Social Structure in the Japanese Queenless Ant, *Pristomyrmex pungens* Mayr

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Pristomyrmex pungens Mayr (Formicidae, Myrmicinae) is a common ant in Japan. This species has no queen and rarely produces males. Unmated workers reproduce by parthenogenesis (thelytoky). I studied two aspects of the social structure of this species.

1. The population structure

Results of inter-colonial transfer experiments and field observations suggested that the ants can discriminate members of their own colony from those of other colonies. Attacks were observed when two colonies came into contact. Thus, populations of this species were considered to be divided into many colonies as those of other social Hymenoptera. The hypothesis that queens produce colony-specific odor as discriminator which contribute inter-colonial discrimination mechanisms cannot be applied at least to this species, because this is a queenless ant. When individuals of two different colonies were artificially intermingled, the substances which serve for colony recognition were transferred between individuals from different origin. Which mechanism operates in this species, the gesutalt model or the operation of environmental odors should be verified in future. Nest relocations (colony movements to the new nest site) were frequently observed and the mean residence time of a nest at a nest site was 16.6 ± 13.9 (mean \pm s.d.) days ($N=239$). There was no apparent periodicity in the occurrence of nest relocation.

2. The structure within the colony

Division of labour was found in workers. Ovarian conditions were apparently different between workers collected inside the nest and those collected outside the nest. Most of the former had well developed ovaries containing mature eggs. Laboratory observations showed that all workers stayed in nests and perform intranidal tasks when they were young. Oviposition was made during the intranidal period. After the intranidal worker stage, they began to perform extranidal tasks (age polyethism). Individuals who became extranidal workers never laid eggs again. There was no aggression among egg-laying workers. However, each worker showed somewhat diverged behavioural ontogenesis, and they could be divided into three types: (1) nurses, which frequently performed care of eggs during their intranidal period, (2) early foragers, who performed extranidal tasks earlier than the other two types and showed low intensity nursing during their short intranidal period, and (3) normal workers, which performed all intranidal tasks at the average frequency of the colony within their intranidal period. In the field, slight but statistically significant differences in body size between intranidal and extranidal workers was detected. The correlation between size or behavioural ontogeny and the fecundity should be verified to determine whether this species is truly eusocial or not.

Queenlessness in *Megalomyrmex* (Formicidae: Myrmicinae), with a Discussion on the Effects of the Loss of True Queens in Ants

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The exclusively neotropical *Megalomyrmex*, ground inhabiting ants of tropical and subtropical forests, includes 31 species divided into 4 groups (Brandão, 1986). The 11 species of the derived Amazonian *leoninus* group have inseminated workers (gamergates) as the only females reproductives, which are extremely "worker-like" in some species and in others are morphologically modified and somewhat physogastric. This study summarizes behavioral observations at the field and under artificial conditions of 3 *Meg.* species (2 queenright and 1 queenless), including comparisons of division of labor. Finally I present the results of an extensive bibliographical survey on queenlessness in ants, with a discussion on the effects of queen replacement by gamergates.

MATERIAL AND METHODS

Field observations and collections were made in three localities: *M. goeldi*, 4 colonies, Petrópolis, RJ, Brasil; *M. iheringi*, 1 colony, Boracéia, SP, Brasil and *M. leoninus*, 1 colony, Cucute, Mérida, Venezuela. Informations on nests, diets and ethograms are given in Brandão (1983).

RESULTS

Queenless colonies occupy spaces among stones or under trunks, never have permanent or rigid nests and migrate frequently. Workers forage individually on the ground or on shrubs, but also tend membracids and do not use the venom apparatus to trail-laying as in queenright species. Gamergates may leave the nest to forage. Ethograms indicate that workers in gamergate containing species are less specialized in roles, do not carry nestmates, lay trails or excavate.

It is often difficult to decide whether "worker-like" females in ants originated from workers that took over the reproductive function or are derived from true winged females through ascending steps of brachiptery, resulting in the loss of wings and progressive modification of other characters. In either case the results are similar. However, in *Megalomyrmex* of the *leoninus* group it can be shown that the female reproductive is a specialized worker with a modified venom apparatus, permitting its insemination.

In the literature I found records of 3 classes of females that depart from the "typical" winged queens. At least in some species of 10 genera (3 subfamilies) there are intergrades between winged queens and workers, indicating that the brachipterous route may have prevailed. In 9 genera belonging to 3 subfamilies true queens have never been found. Other 24 genera in 5 subfamilies possess species or species groups with true queens and others with gamergates, with no brachipterous intermediates, though mated workers can coexist in the same nest with dealated queens.

In *Ophthalmopone*, *Diacamma* and *Rhytidoponera* workers have been observed mating. In *Diacamma* the males had their wings gnawed away, as

in Eciton. Peeters & Crewe (1984) demonstrate in Ophthalmopone that the time of worker emergence determines which are inseminated. This may represent the first step toward the differentiation between "normal" and reproductive workers and brood synchronization.

Protodichthadiiynes of certain Ponerinae, called by Wheeler (1937) "essentially a very robust worker with large ovaries and gaster" may represent a fourth step in the phylogenetic trend toward gaster enlargement of gamergates. Dichthadiiform females of some Ponerinae and all genera of legionary and army ants have the eye vestigial, mandibles falcate and are wingless. It is altogether more probable that they have evolved from gamergates, than by transformation of the winged females.

Colonies of stage 1 species may have up to 40% of their workers inseminated. The phylogenetic trend in morphological specialization of gamergates is accompanied by a reduction in the number of mated workers per colony.

Species with gamergates, irrespective of the particular stage, are characterized by non-permanent nests, frequent colony emmigration, predaceous habits involving primarily termitophagy and myrmecophagy and various degrees of group predation or specialized group-raiding (except Megalomyrmex), more than one insemination of females in their life-time or more than one inseminated individual in the colony, brood synchronization, preference for ground stratum and tropical distribution.

Most formicid societies are characterized by two very distinct behavioral repertoires, i.e., that of the queens and that of the workers. After wing shedding queens in the Formicoid complex show a strong tendency to negative phototaxis and a claustral life. The same is true for Poneroid complex queens after the emergence of the first group of workers. On the other hand, workers do most of the foraging necessary to rear brood and are thus committed to return to their mother's nest. Workers in all worker colonies, even if one or several are inseminated, are not tied to the queen's behavioral repertoire restraints and may adapt their own behavioral catalogs to a drastically changed situation. Without being restricted to live in permanent nests, foraging efficiency may be increased if the colonies can move to new nest sites where food is more abundant. This may be specially important in ground inhabiting predaceous tropical ants. Moreover queenlessness may have been an important factor favoring nomadism in ants.

All nomadic and pre-nomadic ants discussed thus far are tropical or subtropical in distribution. Although nomadism is restricted to the Poneroid complex, this constitute such a diverse taxonomic assemblage that it suggests that the adaptive value of the army ant life style in tropical environment is great.

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Contribution to the Study of Polyethism of *Odontomachus troglodytes* (Formicidae, Ponerinae)

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Odontomachus troglodytes is a typically predatory species equipped with trap-jaw mandibles. It is known that some societies can raise aphids. The worker caste is monomorph, queens are very similar to the workers.

This study is based on 10 societies, each artificially limited to 1 queen, 30 individually marked workers, 20 cocoons and 30 larvae. We studied the repartitioning of the tasks carried out by the first 30 workers and the succession of the roles occupied by the workers which emerged from the cocoons in the artificial nest during 12 weeks.

For the first 30 workers there is a social regulation, where 28% of them go to the exterior service as early as the first week. There are 17.7% which harvest sugary substances and hunt, and 10% which are specialists of predation. From the 200 cocoons at the start we obtained 91 workers and 1 gyne. All of the workers are inactive during the first four days of adult life. During this time their cuticle becomes progressively darker. From the 4th day to the 11th day they move within the nest and do brood care, but it is only after the 11th day that it becomes clear who nourishes the larvae. Some (30%) can eventually leave the nest between the 11th day to 20th day, but never come back with food. During this period there is an apprenticeship of the functions in the exterior service. It is only after the 20th day that there is an evidence of construction and supply activities. Some workers seem to remain inactive during all their life (10%), and some others remain in the interior service as nurses or cleaners (40%). For the passage of the ants from the interior service to the mixed service (20%) we see:

- nurses - cleaners with necrophoric behaviour
- nurses - cleaners and construction activities
- nurses - construction only.

And for the passage to the exterior service (30%) we have only seen:

- nurses - foragers (harvest of sugary substances and predatory behaviour).

Later on, 11% of this type of workers seem to become specialized in predation. The guards are among the workers of the mixed service and of the exterior service; there is also often an intermediary function before the passage to the exterior service.

Preliminary Study of Polyethism in *Brachyponera senaarensis* (Formicidae - Ponerinae)

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The Ponerine ant, *Brachyponera senaarensis*, approaches species of more evolved subfamilies because of an important dimorphism between the queen and the workers, a large number of workers per colony and a partially granivorous feeding behaviour (but they also perform predatory behaviour). This study is based on 9 colonies, each artificially limited to 1 queen, 50 individually marked workers, 20 cocoons and 12 larvae. We studied the repartitioning of the tasks carried out by the first 50 workers and the succession of the roles occupied by the workers which emerged from the cocoons, in the artificial nest, during 10 weeks. In this species, there is a very great difference of length between workers from societies of different places, surely in relation with quantity and quality of nourishment, depending on the habitat. When colonies with small workers produce sexual brood, microgynes form. In the societies with large workers, during production of sexual brood, two kinds of workers are formed which present some differences in their respective age polyethism. The first, originating from small larvae, are produced all year long and have a whitish colour after cocoon emergence. The gaster is small. They are inactive during the first four days, then perform brood care and domestic tasks inside the nest; finally, on the 17th day, some go outside the nest, become foragers and specialized in prey capture from the 19th day on. The second type of workers, produced from large larvae and cocoon, like the gyne and the male, are physogastric and black upon emergence. They are active as early as the first day (brood care), become foragers from the 5th day and are able to capture prey from the 12th day on. Before becoming foragers, all the workers wander about the trophoporic area during several hours or days without bringing back food. They feed on liquid sugary substances before doing activities of gathering seeds or dead arthropods. The predatory behaviour appears to be different from one worker to another (rapidly in few cases, progressively in general). Apparently, no foragers exist that are only specialized in predation.

CONCLUSION

In this species, we have encountered two types of trophic determinism: 1st. difference of the length of the workers from one colony to another in function of the situation of the nest. Nests with small workers produce microgyne and are situated in arid zones. 2nd. Production of well pigmented physogastric workers during the period sexual formation. The latter take up external service very quickly in comparison to the other workers which are not pigmented at the cocoon emergence.

Termite Polyethism

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Integrating mechanisms in a termite society, as in other social insect colonies, are based to a large degree on a division of labor wherein morphologically different individuals (representing different castes, sexes, or ages) tend to be specialized for the performance of particular tasks. The general outlines of **caste polyethism** are well known: a) Reproductives produce the brood; b) soldiers defend the colony against intruders; and c) workers provide care for the brood and other dependents, build and maintain the nest, and forage outside it. Examples of **age polyethism** in the worker line include initiation of nest repair and foraging trails by the oldest workers of *Nasutitermes* species. Examples of **sex (size?) polyethism** include sequential duty by large (male) and small (female) workers of *Odontotermes distans* at swarming site emergence holes, attending of the queen by female workers, and foraging by male workers. In the soldier line of *N. exitiosus*, the small (male) soldiers are aggressive in their defense of the colony, whereas the large (female) soldiers flee from a disturbance. This latter behavior, however, may be important also to colony defense by serving to relay quickly the news of colony invasion.

The consistent behavioral repertoires of the various termite types in a colony reflect differences in capacity due to temporal changes in the activity of exocrine and endocrine glands; to maturation of muscular, nervous, and sensory systems; and to a sequential or differential activation of relevant gene systems. Despite much behavioral consistency, however, the capacity for individualized action remains, and the duties of a termite colony are not rigidly prescribed.

Caste Polyethism during the Process of Food Acquisition in the Termite *Macrotermes bellicosus* (Smeathman)

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Polyethism and processing of food had earlier been analysed in *M. subhyalinus* (S. Badertscher et al. 1983). In general the division of labour was age dependent with only the older workers involved in foraging parties. Gerber et al. (1986) extended these investigations to field populations of *M. bellicosus*. They confirmed the earlier findings and found in addition that at building sites only older minor workers were present, whereas at foraging sites 74% of the termites present were major workers.

Food near the nest is approached by extending a network of subterranean galleries and when food is detected it is covered with soil. We were interested in the question of how the tasks were shared between minor and major workers during the process of gallery extension, food detection and foraging.

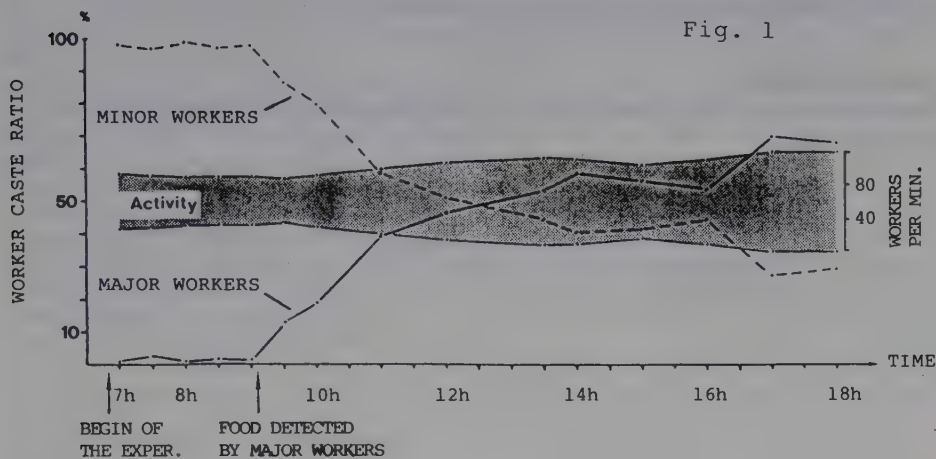
MATERIALS AND METHODS

The experiments were carried out by using an adult *M. bellicosus* colony confined in an outdoor concrete tank. To observe subterranean gallery extension and foraging behaviour an experimental arena of 70x70 cm with a glass bottom was connected to the colony. The arena was filled with a 2 cm layer of soil and a piece of food (wood, 5x5 cm) was placed into the arena. In this way gallery construction could be observed through the bottom. For detailed behavioural observations a smaller (16x21 cm), 3 mm thick horizontal glass chamber filled with soil was used instead of the arena.

RESULTS

After connection to the nest a network of radial galleries was extended by the termites into the arena. During this building period nearly all the workers participating were minor workers (Fig. 1). Building behaviour consisting of removing and depositing soil particles was performed repeatedly by single individuals without local fidelity within the network of galleries. After one gallery had reached the food the building individuals involved exhibited local fidelity with reference to this gallery. After the food source had been discovered it was hardly exploited by the

PASSAGES TO AND FROM FEEDING AREA



minor workers and they did not give rise to any recruitment. The gallery branch leading to the food was not widened at this stage. When one of the major workers accidentally came across the food source it normally bit off a piece and tried to transfer it to a minor worker who then carried it back to the nest. After several repetitions of this behaviour and when major foodfinders had returned to the nest increased numbers of major workers were recruited directly to the food (Fig. 1). The larger the number of major workers involved the less foodcarrying by minor workers was observed. When intensive foraging was going on, the gallery branch leading to the food was extended by the minor workers. Major workers rarely participated in building behaviour.

We report for the first time a highly caste specific division of labour during the process of food acquisition with minor workers being involved in building activities and major workers in foraging.

It is of special interest that major workers recruit their own caste and direct them to the food even when this gallery branch is only a minor side gallery of the network. We suspect that this caste specific information is transmitted via pheromon trails.

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Polyethic Structure of the Foraging/Guarding System of Red Wood Ants (*Formica* s. str.)

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Evolution of ant species can, through selection acting on extended phenotypes of queens, perfect the trophic machinery represented by the integrated activity of workers. There is reason to believe that maximization of the net energetic yield of the colony as a whole may conflict with optimal foraging as measured at the individual level of behaviour. The foraging system should match the distribution of resources, including resources yet to be discovered, and a forager searching for new food sources will, as an individual, appear uneconomical compared with a forager visiting a "known" source of food (cf. Deneubourg *et al.*, 1983). Another factor is that ant species which monopolize their habitats have to allocate workers to defend both profitable and less profitable sectors of the territory and hence cannot optimize short-term resource exploitation. Recruiting signals are evidently of immense importance in opportunist species utilizing ephemeral sources of clumped food, but may play a smaller role in territorial denizens of stable habitats using scattered booty and/or spatially persistent resources. It is my thesis, exemplified by red wood ants of the subgenus *Formica*, that such species may be characterized by very "close" habitat relations based on spatial, temporal and functional polyethism among workers.

RESULTS AND DISCUSSION

Red wood ants are clearly territorial, although polygynous populations often jointly defend a group of nests rather than a single nest against competitors, mainly other ants (Rosengren *et al.*, 1986). Monopolization of the habitat by territorialism implies protection not only of present but also of future resource availability, a strategy connected with great colony longevity. In contrast to newly recruited foragers, old foragers or veterans maintain a strong and persistent route fidelity/site allegiance (Rosengren, 1977). The latter feature has been interpreted as indicating the predictive power of past rewards (Rosengren, 1977), but the latter argument is weakened by evidence of high turnover of aphid colonies in forest trees (unpubl.). It could be that the strong site allegiance of veterans does not primarily guarantee prognostic location of food sources, but rather restoration of the territory after periods of dormancy.

It is also plausible that route fidelity/site allegiance represents a colony-level probabilistic tactic for dispersal of searching efforts, since individual-level techniques of random search, although efficient within restricted hunting-plots, cannot cover the large, densely vegetated areas, utilized by colonies of this species group. The resulting "spider's web" of branching routes and permanently occupied extranidal sites is comparable to a huge, collective trap for scattered, spatio-temporally unpredictable booty. This dispersal tactic does not preclude age-correlated social recruitment to new sources of clumped food.

The size-frequency distribution of ant workers of a colony is often assumed to match the size distribution of the booty insects, but this does not agree with the observation that Formica species with approximately similar diet may have very different types of size-frequency curves (Schmidt, 1974, Rosengren et al., 1985). Some species show a weak correlation between forager size and foraging task but only one clear generalization can be made at present. A positive correlation between forager size and foraging distance has been confirmed for several Formica species (Horstmann, 1972, Rosengren et al., 1985, Sundström, this volume). This, as pointed out by Sundström, could increase the ergonomic efficiency of loaded foragers. An additional aspect is that ants foraging far away from the nest may receive less help from nest-mates and certainly have a much higher probability of encountering contest competitors than workers foraging close to the nest. It may thus, for several different reasons, pay to be large and strong when far away from home.

Indirect evidence suggest that the age distribution could influence the size-frequency distribution through differential mortality and/or differences in maturation time from intranidal to extranidal work between workers of different size. Forager age appears in addition to be positively correlated to diligence of foraging because young first-year foragers depart from the nest mainly during optimal conditions whereas veterans forage in high frequency also in cold weather (Rosengren, 1977).

The rules regulating spatial and functional differentiation of the worker force appear, in spite of a statistically consistent pattern, probabilistic rather than deterministic. Noise may be adaptive if matching environmental uncertainty (Deneubourg et al., 1983) but it could also be that sociobiological systems on that large phenotypic distance from the DNA-code are doomed to be flimsy.

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Size Allocation and Task Preference in Red Wood Ants (*Formica*)

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Morphological diversification in workers of social insects may reflect a division of labour enhancing colony efficiency, by matching resource distribution patterns (Oster and Wilson, 1978) and the constraints of central place foraging (Orians and Pearson, 1979).

MATERIALS AND METHODS

Colonies of two *Formica rufa* group species and *F. uralensis* were studied. Samples from the nest surface, all tree climbers and honeydew carriers at different distances from the nest were measured separately using head width as a parameter of size. Comparison of thorax- and tibial lengths to head width yielded isometric relationships. Returning honeydew carriers and departing individuals were also separately weighed and measured.

In one of the colonies were individuals climbing trees also marked according to their activities, carrying honeydew or prey, to assess a possible task preference.

RESULTS

The results show an allocation of the worker force within the existing size range in the studied species.

Proximal individuals were in all cases significantly smaller than distal ones. The territory can be exploited more efficiently and foraging distances can be increased because large honeydew carriers bring in more honeydew per capita than small ones.

Among the tree climbers a task preference was observed, as well as a group of idle individuals, consisting presumably of young foragers still without a definite task preference. These might function as a reserve at sudden bursts of food, as well as defending and patrolling the territory.

If the task preference is supposed to be the consequence of site allegiance, it would still not explain why proximal honeydew carriers were significantly smaller than total samples from the same site. The difference, however, declined with increasing distance from the nest.

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Effects of "Short-Day Treatment" on Honey Bee Polyethism (*Apis mellifera*)

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Cherednikov (1) showed in Leningrad, USSR, that experimental shortening of the daylength in summer leads to a higher rate of cannibalism and to an enlargement of the fat body - both phenomena, connected with the appearance of winter bees. We conducted similar experiments in Berne, Switzerland, in order to see if this phenomena can take place also in middle Europe (2).

MATERIALS AND METHODS

From May until August the natural photoperiod was reduced by a dark box, fixed at the hive entrance. A movable lid was opened between 9 and 11 o'clock in the morning in order to stimulate daylengths of October, November and December. Two other colonies were used as controls: one without box and the other with box with windows. The total lipid content of the fat body of single bees was determined by ether extraction and its soluble protein content by the Bio-Rad method. Determination of the number of brood, the number of bees and the length of life was carried out after (2).

RESULTS

There was no significant effect of "short-day treatment" on the number of brood cells, the number of adult bees and the length of life of workers. On the other hand shortening of the photoperiod led to a significant increase ($p < 0.05$) of the lipid- and protein content of the fat body. This increase is typical for the change of summer to winter bees (3). Thus, shortening of the photoperiod could not trigger the building of long-living winter bees, inspite of its effects on the fat body of workers.

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Rhythmical Patterns of Activity within the Nests of Ants

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The ecological success of social insects is often attributed to the inherent efficiency of colonial life. For example, eusocial workers can perform essential tasks in parallel rather than serially as a solitary wasp must do. Concurrent operations should promote reliability (Oster and Wilson, 1978). However, efficient parallel processing is only possible if there is communication between production lines and reallocation of workers as requirements change. This could only be achieved if workers frequently interact to collate and update information on the status of important tasks. There are, however, few studies showing communication between one worker and many in the context of "household tasks". Important exceptions are the nest reconstruction behaviour of termites (Bruinsma and Leuthold 1977) and between-caste aversion in Pheidole (Wilson 1985).

In this paper I report the discovery of a process that by enabling workers to exchange accurate and timely information may underpin the overall control of operations in the nests of ants. Most ethogram and time budget studies do not reveal patterns of communication within the nest population as a whole because they are based on independent records of individual workers. The following study was therefore undertaken to examine simultaneously the behaviour of all, or the majority, of workers and reproductive females in the nests of the ant Leptothorax acervorum.

METHODS

Colonies of the small insectivorous ant Leptothorax acervorum were collected in Sweden. Four small colonies were each housed under controlled conditions of temperature and low light intensity in nests made from microscope slides and filmed continuously for several days using time-lapse video recording equipment and a camera mounted on a dissection microscope. The colonies had worker populations ranging from 34 to 43 individuals: one colony was monogynous, one had 2 queens, one was queenless and the last had 1 host queen and 2 females of the parasitic inquiline Leptothorax kutteri. The video recording of each colony, from the second day of each filming period, was analysed for the 10 hours of the diurnal period beginning one hour after lights-on. Data was recorded for all the queens and parasites in the nest plus randomly selected workers to give 20 individuals in total. The position of each of the selected ants was marked on an acetate sheet attached to the video monitor, during a particular freeze-frame of the recording as indicated by the video recorder's time date generator. At the end of each minute throughout the 10 hour sample period the state of each ant was recorded as active or inactive. An ant was 'inactive' if it was completely stationary or only made small movements of its antennae; all other behaviour was recorded as 'active'. When a worker left the nest during the sample period it was

replaced in the record by the next worker to enter the nest. This procedure generated a time series consisting of 12,000 records for each nest.

RESULTS

Standard time series analyses were used to detect trends, rhythms and cycle periods in the activity records. If workers were active independently, the frequency at which n individuals are active at any one time would have a binomial distribution. For each colony the observed variance of the activity-frequency distribution was compared with the variance of the binomial calculated from the empirically estimated probability of one ant being active or inactive at any one time. In all cases the null hypothesis was rejected at $p < 0.001$. Autocorrelograms also indicated that the activity of the sampled individuals within each nest was highly synchronized. Spectral analyses showed that activity was roughly periodic, cycling at 15.6, 16.0, 22.3 and 36 minutes depending on the colony. Log-survivorship curves of both activity and inactivity bout lengths for each colony showed a tendency for the termination of each bout to depend, in part, on when it began. The inactivity bout lengths of all 4 colonies were particularly regular with means between 14 and 20 minutes. No significant trends were found in any of the series showing that each colony tended to maintain its own particular rhythm throughout the 10 hour period (Bryant and Franks in prep).

DISCUSSION

As in many other ants, workers of L. acervorum are inactive for approximately 75% of their time within the nest (Oster and Wilson 1978). Long periods of inactivity may conserve energy, but unless the relatively short, activity bouts of workers are synchronized only a few could interact at any one time and the information they exchange would tend to be both inaccurate, due to small sample sizes, and out of date. However, an individual's decision, for example, to forage or exchange food with nestmates should depend not only on accurate information on its own nutritional status but also on that from many other individuals. This might explain why the workers of L. acervorum, as well as their queens and parasites tend to be active in synchrony so that the vast majority of the social interactions and communication in these nests occur in distinct bouts at regular intervals. In this way individuals may be able to exchange reliable regularly updated information on the status of essential tasks within the nest.

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Hormonal Regulation of Age Polyethism in *Apis mellifera*

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Despite the prominence of division of labor in honey bee social organization, the means by which it is regulated are poorly understood. Labor allocation in the honey bee society is characterized by two features: workers display elaborate age polyethism, but are also able to respond to changing colony needs by altering the trajectory of their typical age-dependent behavioral development. One approach to elucidating how the activities of workers are integrated into an efficiently operating colony is to first identify physiological mechanisms that underlie age polyethism. In this paper I summarize evidence supporting the hypothesis that the rising titer of juvenile hormone (JH) associated with the aging of the worker honey bee is involved in the regulation of age-dependent division of labor (for more details, see Robinson, in press).

MATERIALS AND METHODS

I treated individually marked 1-day-old adult bees with the JH analog methoprene, introduced them to typical colonies housed in Langstroth or observation hives and studied their physiological and behavioral development. Methoprene is used extensively as a JH analog because of its proven JH activity in all insect species on which it has been tested, including the honey bee. In most experiments, methoprene doses were administered from coded vials, allowing observations to be made blind.

PHYSIOLOGICAL AND BEHAVIORAL EFFECTS

Bees treated with 250 μ g methoprene began foraging on average 8 days earlier than untreated individuals, and subsequent experiments have demonstrated that premature foraging can be induced in a dose-dependent manner with applications of 50 to 200 μ g. Methoprene also induced premature production of the alarm pheromones 2-heptanone and isopentyl acetate. Methoprene's effect on 2-heptanone production is consistent with its causing premature foraging, because the onset of 2-heptanone production is correlated with the beginning of foraging. Isopentyl acetate production typically begins at 3 to 4 days of age; its onset is not associated with the nest-to-field shift. Premature synthesis of isopentyl acetate by methoprene-treated workers suggests that JH is also involved in the regulation of developmental processes that occur prior to foraging, and may also influence the expression of age polyethism in the nest.

Evidence supporting this hypothesis was obtained. Methoprene exerted dose-dependent telescopic effects on the age polyethism schedule: there were significant decreases in the number of days engaged in brood and queen care, food storage, and nest maintenance, and a premature onset of foraging. Treatments also significantly affected the age-dependent frequency with which acts of brood and queen care, food storage, and nest maintenance were performed, again in a dose-dependent manner.

MODEL EXPLAINING ROLE OF JH IN REGULATING DIVISION OF LABOR

Based on these results a model of the mechanisms controlling division of labor was developed (Fig. 1), which indicates that as JH titers change they modulate sensitivity to odors associated with the various tasks in honey bee nests. The effects of methoprene on pheromone perception are consistent with the model: treatments prematurely reduced the behavioral threshold sensitivity to alarm pheromones. According to this model, JH acts

as a behavioral primer, regulating the colony's allocation of labor by altering the probabilities with which individual bees perform certain tasks. The model also predicts that regulation of JH titers by environmental and colony conditions underlies the workers' ability to respond to changing colony needs.

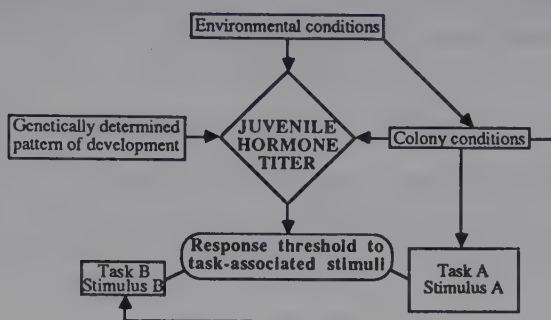


Fig. 1. Model explaining the role of JH in regulating honey bee division of labor. The probability that a worker bee will perform a given task is determined by: 1) JH-dependent response thresholds that determine the probability of responding to stimuli that elicit the performance of a task, and 2) environmental and colony conditions that determine the probability of encountering a task, by shaping colony needs and determining the relative magnitude of the tasks (depicted here as the relative size of "Task A" and "Task B" boxes). According to the model, JH titers increase with worker age due to a genetically determined pattern of development; in addition, JH titers (and therefore, response thresholds) may be modulated by environmental and colony conditions, enabling individual workers to respond to changing colony needs.

CONCLUSIONS

The present case for JH's involvement in age polyethism rests largely on the results of treatment experiments with methoprene (see also Jaycox et al. 1974; Fluri et al. 1982). Other studies suggest that the high doses of methoprene used relative to endogenous JH titers indeed induced physiologically relevant responses. Nevertheless, correlations between JH titer and behavioral status, if possible, would provide important supportive evidence for JH's role in regulating age polyethism.

JH's role in the differentiation of morphologically distinct castes of social insects is well known (Nijhout and Wheeler 1982), but the honey bee is the first species in which a hormonally mediated system of temporal caste determination among workers has been described. Given the pervasive role played by JH in morphological caste determination, hormonal regulation of temporal caste structure may also be widespread.

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Individual Behaviour and Polyethism

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One of the fundamental criteria for eusociality in insects involves the division of roles between members of the society. A result of this polyethism is the existence of functional groups, which are distinguished by their specialisation in different tasks. The social organisation of different species is primarily characterised by the number of individuals in each functional group and the proportion of their specific activity with respect to the entire colony's involvement in the task. This specialisation may be a result of interindividual differences based either on morphological features in polymorphic species or more generally on maturational factors that are associated with age.

This age-linked internal physiological state is however not the only factor responsible for the variability of behavioural expression observed within societies. Indeed, same-aged workers, that presumably are in the same physiological state, do not necessarily react in the same way to an apparently identical situation. This was the case in an experiment reported by Jaisson et al. (1986) in which 50 Formica polyctena workers, from the same colony and of identical age and size, were isolated with only cocoons and food two hours after emergence. Under these conditions, the behavioural response to a stress test inducing cocoon transport revealed considerable variability between individuals as the timing of appearance of the first responses.

We used a marking method for individually identifying each worker, which allowed us to determine the inter-individual variability within the society. This has the advantage of being a natural situation in which the individuals are subjected to the social pressure of the other workers.

This method was used to investigate a neotropical ponerine ant, Ectatomma tuberculatum, which presents a sensitive period during the first four days of social contact (as opposed to post-emergence life) for the development of brood-care activities (Champalbert, 1985). From the experimental groups submitted to a 10-day period of isolation at different post-emergence ages, the only group that showed a severe impairment of this behaviour after re-insertion into the colony was the one which was isolated between 2 and 12 days. All of the individuals of the impaired group did not however react in the same way. Despite the fact that they were of the same age and were subjected to the same experimental conditions as the impaired ants, certain workers not only show normal ethogenesis of this behaviour, but become true "nurses" with all of the characteristic behaviour patterns of this functional group. One is thus led to conclude that the sensitive period is reduced from the usual four days to only two days for this group.

Other studies have also shown that the phenomenon of inter-individual variability is not simply an artefact associated with isolation. In contradiction to the predictions that should ensue from control by age polyethism, same-aged workers may present different functional spe-

cialisations. Thus, *Ectatomma ruidum* workers may be either "inactive" or "larvae-nurses" when aged between 0 and 5 days, and either "larvae-nurses" or "house-maids" between 6 and 10 days (Corbara et al., 1986). The same type of result is obtained for *E. tuberculatum* workers, which at a given age may be either "nurses" or "intermediates" or else "inactives" (Champalbert, 1985).

One often tends to consider the functioning of a society on the basis of the activity of averaged individuals, which may easily be interchanged within the society's various functional groups. As a general rule, however, individual variability results in heterogeneous distribution of workers in the different functional groups, even though the average of the constituent members of the groups provides sufficient common characteristics to justify their pooling on the basis of computerized statistical analysis (see Corbara et al., 1986). Within each functional group, an analysis of each individual's degree of specialisation revealed a continuous distribution ranging from highly efficient to inefficient individuals (Corbara et al., 1986). This phenomenon appears at present to constitute a key factor for providing a partial explanation for the regulatory mechanisms that allow social insects to maintain their social organisation in balance. Indeed, when this balance is upset (for example, as a result of the disappearance of a functional group), social regulation is achieved as a result of individual responses that depend on a certain "priming" for the task to be regulated. Similarly, in the case of foundations, the first workers may be "pre-disposed" to accelerate their age polyethism. For each individual, this "priming" may be a result of an integration of its physiological state, its previous experience, social pressure, and finally, of competition between individuals, for access to the given task (Lachaud and Fresneau, 1986).

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The Genesis of an Ant Society: a Natural Model for Social Regulation

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The investigation of social specialisations of members of a society, from the foundation by the queen until the society attains maturity, provides a remarkable illustration of the process of ergonomic integration of the first workers into the collective activity of the colony. As opposed to experimental sociotomies in the laboratory, these changes are gradual and do not produce abrupt variations of social parameters. This study is therefore of interest for the investigation of the ontogeny of a society as a natural model for studying social regulation.

A society of *Ectatomma ruidum* Roger, a neotropical ponerine ant, was therefore recorded over 8 developmental periods: solitary queen, and then accompanied by 3, 4, 5, 7, 18 and 27 workers respectively. The results of an automated photograph method recording (Corbara et al., 1986) reveal that the characteristic equilibrium maintained by adult colonies is obtained as early as the 7-worker stage. The use of an hierarchical classification technique allowed us to identify 4 groups of workers for each period, each associated with a functional specialisation: egg nurses (comprising only the queen), larvae nurses, intermediate-inactives and guardian-foragers. Whereas the queen's behaviour undergoes considerable modifications, the larvae nurses and foragers display, on the contrary, a remarkable stability of their specialisation profile, from as soon as the 3-worker stage. This may be due to the high degree of specificity of these tasks.

Age polyethism is certainly involved as a frame-work leading to this species specific social division of labour. *E. ruidum* societies are characterised by having few workers specialised in foraging and many participating in egg- and larvae nursing and in inactivity. According to the society's developmental stage, the arrival of new workers results in a re-inforcement of one or other of these functional groups. This re-inforcement occurs, in this instance, as a result of a partial blockage or a slowing down in the passage from one stage to another in the different specialisations that constitute the specific age polyethism.

Age polyethism thus would primarily involve the organisational factor resulting in the specific chronology of functional specialisations leading to the structuring of social behaviour. The specific behavioural profile involves both the proportion of the total activity represented by a given task and the number of individuals that are in the group specialised for that task. These features would act as modulators of the basic pattern defined by age polyethism. Such a mechanism, of course, pre-supposes a considerable degree of individual behavioural plasticity. Such an assumption appears justified upon examination of the evolution of the individual behaviour of the first workers.

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Age-Polyethism in Stingless Bees and Evidence of Flexible Individual Ontogenetic Sequences

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The behavioral repertoire of worker stingless bees includes some typically specialized worker activities. Their characteristic mass provisioning system of larval feeding has led to the evolution of specialized food dischargers. In some species these provisioners have extremely swollen abdomens. A very limited number of such food discharging specialists can supply the cell in a very short time with all the larval food. This contributes to the typical rhythmic occurrence of cell-provisioning-and-oviposition bouts. Specialized laying worker behaviour is another stingless bee characteristic. However, it does not generally occur in all species.

MATERIAL AND METHODS

Several series of observations were carried out on colonies of *Melipona favosa*. All the bees of a nest were individually marked after emergence and studied during: brood cell construction; cell provisioning; worker oviposition; guarding; waste-dump activities; foraging; court activities; pollen uptake at storage pots. We studied colonies with continuous emergence of young bees as well as colonies with interrupted brood production.

RESULTS

In addition to the distinct age-dependence for performance of most nest behaviours there is a remarkable individual correlation between larval feeding and cell building. Furthermore, the bees which participate most actively in the court interactions (with the queen) are also the ones which are on that day the most active in the construction and the provisioning of broodcells (Sommeijer, 1984; Sommeijer et al., 1985). Pollen feeders belong to a wide age group (1-48 days of age), most frequent feeders being 14-27 days old. We studied the relation between the oral pollen uptake in the pots and the nearest day of broodcell provisioning. The most active pollen feeders had provisioned broodcells 10 days before their recorded pollen uptake. As in other social insects, foraging is the final worker duty. Nectar and pollen were collected by bees of the same age (24-64 days old). Only 25% of the observed foragers (three months observation) collected both nectar and pollen. When they did so, this was done in separate flights. There was no consistent pattern in the succession of foraging. This is in contrast to the definite ontogenetic sequence performance observed in nest activities.

Flexibility of the age-based division of labour can be observed particularly in the discharging of larval food. In colonies with regular emergence of young bees, larval food dischargers are about 10-12 days old and participate for a period of only two to three successive

days. However, in colonies where the emergence of young bees is interrupted, workers may, after discontinuing their discharging, resume this after a few days. Such bees may remain involved in provisioning for more than 40 days.

The laying workers demonstrate remarkable variation in the age at which they lay their eggs, in their behaviour during oviposition, and in the type of egg that is released. We found that the development of a specific type of laying worker behaviour depends primarily on queen dominance variables: dominant queens cause their workers to lay defective eggs. We can now make some comments on the ultimate function of this typical organization of worker behaviour. The characteristic division of labour for within-nest activities, which includes the typical correlation of queen-interaction tasks with brood production activities, probably results from the evolution of a typical system of queen dominance in stingless bees. The behavioural feature whereby cells are provisioned, oviposited and operculated in the course of a very short period of time, could be an adaptation to an attack inside the nest by natural enemies like phorid flies. It is remarkable that not only broodcells are immediately closed, but also the pollen pots, even if these still contain very small amounts of food.

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Ecological Correlates and Behavioral Flexibility of Temporal and Physical Castes in the Ant *Pheidole dentata*

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Caste theory states that the age and size frequency distributions of workers have been selected at the colony level and are adaptive. This model of adaptive demography assumes a tight correlation between caste and task performance such that caste membership determines behavior. However, several studies have shown that behavior may be flexible, and that workers adjust their labor according to colony needs. Recently we have shown that in the ant *Pheidole dentata*: 1) temporal and physical caste ratios are highly variable, and are not correlated with ecology or reproductive output; and 2) the tasks performed by minor workers of different ages change in response to colony demography.

The ecology of temporal and physical castes

Foraging and defense are, respectively, age- and/or size-related tasks in the dimorphic ant *Pheidole dentata*. Therefore, colonies in different trophic, competitive or predatory environments are predicted to have different worker age and size frequency distributions. We examined the association between those demographic distributions and ecological parameters in populations of colonies in significantly different habitats in northern Florida. The habitats were chosen because they differed significantly in drainage, soil type, and vegetation age and composition. Based on ten months sampling over three field seasons, they were shown to differ significantly also in ecological factors most likely to influence caste and social structure: availability of potential food (invertebrate biomass), distributions of and interactions among ant species, and intensity of anuran and mammalian predation (Calabi and Traniello 1986a). Notably, a major competitor, *Solenopsis invicta*, was present in only one habitat. To determine whether these ecological differences affected social organization, 42 whole colonies were collected from the two habitats and the numbers and proportions of adults in each physical caste were censused. In 30 of these colonies the minor worker age castes were also censused. No single adult physical caste, nor any ratio among castes, nor colony size based on any summed combination of castes, was correlated with habitat (Calabi and Traniello 1986b). Thus, in spite of differences in the competitive environment, and in the presence of vertebrate predators and *Solenopsis invicta*, there were no differences among colonies in the proportion of majors. There were also no differences in the number or proportions of alates produced in these colonies. In the 31 colonies in which minor worker age castes were censused, no age caste alone, nor any ratio of age castes was correlated with habitat. Although colonies were collected from habitats with different trophic environments, there were not proportionately more age caste III, IV or V individuals (typically foragers) in colonies from the habitat with greater food availability. There were great differences among age

caste ratios from colonies within as well as between habitats, and even among colonies within each two-week sample period per habitat. Following censusing, ethograms were constructed for each colony to reveal patterns of polyethism. Although some colonies had very different caste ratios, their task performance profiles were quite similar.

Flexibility of the age/task association

Flexibility of division of labor among minor workers was studied in colonies with experimentally altered age caste ratios. Based on comparisons among behavior performances, in colonies without young workers, old workers performed proportionately more inside nest tasks than did their same age-peers in control colonies, and young workers in colonies without old workers foraged significantly earlier than did their age-peers in control colonies. Also, in colonies without young workers, old workers were found in spatial association with brood proportionately more often than were control age-peers. Conversely, in colonies without old workers, young workers associated proportionately less with brood than did control age-peers. In sum, minors of the ant Pheidole dentata were highly flexible in their performance of brood care and foraging behaviors.

Discussion

P. dentata may be characterized more by variation in physical and temporal caste ratios than consistent demography. Moreover, the behavioral development of a worker is influenced by colony demography as well as by the worker's own age and caste. Selection over evolutionary time for relatively coarse species-typical demographic patterns may have occurred, but environmental unpredictability may have favored behavioral flexibility to permit workers to meet immediate colony needs. Our results suggest that colony labor or behavior profiles cannot be assessed from caste ratios alone. In particular, tests of the adaptive demography hypothesis must include comparisons of both demographic distributions and the observed, rather than inferred, labor profile.

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Determinants of Social Structure in the Ant *Novomessor albigetosus*

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Social insect colonies are composed of castes, or groups of specialist workers which can concurrently perform different tasks with high efficiency (Oster and Wilson 1978). But the phenotype of a colony is more than the sum of its castes: detailed information on the behavior of known individuals is necessary for understanding the dynamics of colony behavior and social regulation.

We conducted ethogram observations on marked workers of *N. albigetosus* and examined patterns of task performance at the individual and colony level. Behavioral flexibility was tested by removing larval care specialists, observing replacement workers, and returning the specialists after 10 days. The importance of learning in developing task specialization was tested in isolated groups of larval care specialists and their replacements. Efficiency in rearing larvae was indicated by frequency of larval care acts and by larval weight gain.

Three outstanding aspects of social structure were found. While *N. albigetosus* exhibited a typical pattern of temporal polyethism (McDonald and Topoff 1985), it was also true that except for the oldest workers, which never tended brood, workers of all age classes performed all tasks at least occasionally. Workers exhibited great variation in frequency of task performance and degree of specialization on specific tasks. Up to 8% of the workers in a colony performed no tasks at all. The number of specialists for any one task is small relative to the total number of workers that perform the task.

When specialists in larval care were removed from a colony they were quickly replaced. After 10 days some replacement workers acted as specialists, even after the original specialists were returned. Both behavioral data and larval weight gains indicated that isolated replacement workers were one third as efficient as specialists during the first 3 days, but nearly as efficient after 10 days.

The replacement of specialists indicates that specialization is socially regulated: specialists inhibit the development of further specialists, possibly by simple monopolization of task opportunities, depriving other workers of positive feedback. The slow development of new specialists suggests that learning may be involved. Efficiency in rearing larvae increased with experience in isolated workers, supporting this hypothesis.

It may be useful to view an ant colony as a few workers which have "differentiated" into specialist roles and many "undifferentiated" workers. This could relate to flexibility of responses at the colony level.

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Learning Abilities of Ants and Complexity of Social Systems

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Ant species with communication systems of increasing complexity, as previously classified (Jaffe, 1984), were investigated in order to assess any relationship between social behavioural complexity and learning ability.

MATERIALS AND METHODS

Cues used for orientation were evaluated as follows: A Y-shaped maze was connected to the foraging area of the colony until an ant walked into it and emerged again from an opposite arm of the maze into a plastic container. The maze was immediately removed from the colony, leaving the ant isolated for 20 min, after which the maze was offered to the ant. "Homing" choices were recorded. The effect of visual, olfactory and spatial stimuli upon homing were tested by recording from a foil-paper-covered maze, a new maze, or when offering any arm of the maze (s, -s) to the isolated ant, respectively.

The learning ability was tested by placing marked ants into the maze and recording the choice. When "correct", the ant was reinforced by placing it back to the nest for at least one hour; when "incorrect", the ant was punished by isolating it for 10 min before reintroducing it into the maze. A new maze was used for each trail.

RESULTS

Table I shows the effect of different cues upon the correct homing of the studied species. All values above 67% or below 30% were statistically significant ($p < 0.05$, binomial test, $n=20$). This allowed the assessment of the relative weight of the various cues used for homing (Table II). These were classified as visual cues (v), including visual landmarks, shadows, etc.; olfactory cues (o), which included the topochemical sense, tactile stimuli, etc. and spatial cues (s, -s) which referred to a right-left turn memory. The learning ability in the presence of visual cues is shown in Table III. Species showing higher learning rate were T. urichi, E. ruidum and A. laevigata, as values above 80% were statistically significant ($p < 0.01$, binomial test, $n=20$). The first two species are those with the simplest social system among the species studied, whereas A. laevigata is the socially more complex species.

TABLE I: Effect of visual (v) olfactory (o) and spatial (s,-s) cues upon the percentage of ants homing.

Species	1	2	3	4	5	6
Cues						
v,o,s	100	94	75	90	85	74
o,s	75	64	80	68	35	75
v,s	63	69	80	60	72	81
v,o,-s	65	84	65	80	80	22
o,-s	65	68	70	87	60	29
v,-s	80	62	60	-	80	22
s	50	48	47	54	-	73

TABLE II: Relative weight of cues used for homing.

Species	1	2	3	4	5	6
Relation						
v-o	+	-	-	-	+	0
o-s	0	+	+	+	0	-
v-s	+	0	0	0	+	-
s-(v+o)	0	-	0	-	-	+

TABLE III:

Learning ability (%) of correct choices in 3 consecutive trails.

Species	1	2	3	4	5	6	1* 3*
Trail							
I	35	43	45	37	40	55	40 55
II	40	39	70	58	40	55	35 55
III	80	61	60	81	80	65	60 65

Species are: 1:Atta laevigata, 2:Acromyrmex octospinosus, 3: Acromyrmex rugosus, 4: Trachymyrmex urichi, 5: Ectatomma ruidum, 6: Pseudomyrmex termitarius. In Table III, species 1-6 maze with visual cues, * without visual cues.

CONCLUSIONS

- Each species uses more than one cue for orientating during homing.
- Each species uses the different cues in a different hierarchy.
- The learning ability for homing, using a certain cue, is different for the various species and do not seem to be related with the specific hierarchy of cues used by the species, in a direct and simple way.
- The ability to learn seems to be inversely related to social complexity, i.e., T. urichi and E. ruidum, the socially more primitive species, learn much faster than the Acromyrmex spp. which have a more complex communication system, even showing polymorphism. A. laevigata the highly polymorphic species, shows a more complex pattern.

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Colony Age and Organizational Flexibility in Harvester Ants

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Harvester ant colonies do various tasks outside the nest, including foraging, nest maintenance, patrolling, and midden work. These activities are done each day in a characteristic sequence, leading to predictable temporal patterns in the numbers of ants engaged in each activity. Perturbation experiments in the field show that different tasks are interdependent: single perturbations affecting only foraging, nest maintenance or patrolling cause changes in the temporal patterns of other tasks. Effects of combined perturbations are non-additive, showing that colonies re-order task priorities in response to increasing levels of environmental stress. In perturbation experiments comparing colonies about 2 years old with ones about 5 years old, there were two main differences: older colonies respond more consistently to the same perturbations in successive experiments, and older colonies show more non-additive effects; in particular, older colonies decrease foraging less as stress increases. Since individual ants live only about a year, these age differences are the result of ontogenetic changes in colony organization, not the accumulated experience of particular, older individuals.

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1.4.2 Nest structure

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The Combs of Honeybees as Composite Materials

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In addition to the metabolic costs of secretion, wax imposes material constraints on bees. Some physical and chemical properties of the starting materials are known (cf. Hepburn 1986), but the ways in which the complete nest changes with time and temperature have not been studied. We report results of measurements of some of these changes.

MATERIALS AND METHODS

We used combs of *A.m. adansonii* that ranged in colour from white to brown and that varied in silk content. Tensile tests were performed on chloroform-extracted sheets of cell wall silk, silk-free white comb wax, and brown cells walls of 34% silk content. Tensile tests and the analysis of data were performed as previously described by Kurstjens et al (1985).

RESULTS AND DISCUSSION

The development and maturation of brood comb proceeds from a single-phase material, pure wax, to a fibre-reinforced composite. Fibroin, a crystalline and hygroscopic protein, is spun randomly on the cell walls so that the intact walls exhibit planar isotropy. Honeybee fibroin is mechanically constant between 25° and 45°C for stiffness, strength and distensibility. (Table 1)

TABLE 1. Tensile mechanical properties of dewaxed sheets of honeybee cocoon silk. (for each value $n = 10$)

Temperature °C	σ (Nmm ⁻¹)	ϵ (%)	E (Nmm ⁻¹)	Work (MJm ⁻³)
25°	32 ±16	98	33 ±14	29 ±20
30°	32 ±18	81	40 ±13	28 ±23
35°	26 ±10	85	31 ± 8	22 ±14
40°	39 ±17	105	37 ±14	38 ±22
45°	43 ±20	106	41 ±14	48 ±30

The beeswax of comb is an isotropic plastic having no unique mechanical constants: stiffness, strength and extensibility all vary with the rate of deformation and with the temperature (Hepburn et al. 1983, Hepburn and Kurstjens 1984). Silk-free comb walls of white wax, for example, exhibited an order of magnitude difference between 25 and 45°C. Such wax failed plastically at ever lower extensions with increasing temperature (Table 2). Similarly, stiffness and fracture energy decreased as the temperature rose (Table 2).

The tensile behaviour of brood comb, a two-phase composite material, is well illustrated by comparing the walls of silk-free white

comb with similar specimens of brood comb having a substantial amount (34%) of silk (Table 2). Between 25 and 45°C, both preparations decreased in strength and very rapidly so, near 40°C. The fibre reinforcement of silk in the propolis-bearing brown comb was an order of magnitude stronger than white wax alone; the former was considerably stronger than the latter at all of the assay temperatures. On average, the breaking strain in the reinforced comb was double that of white wax (Table 2). Combined with the greater loads required to break combs reinforced with silk, this greater distensibility provides a safety factor of 1 over that of white comb wax.

TABLE 2. Tensile mechanical properties of white comb wax walls with no silk and brown comb walls of 34% silk. (for each value $n = 6$)

Specimen	Temperatures °C	σ (MPa)	ϵ (%)	E (MPa)	Work (MJm ⁻³)
white comb wax	25°	1.0 ± 0.1	12 ± 1	9.1 ± 0.1	7.4 ± 1.7
	30°	1.5 ± 0.4	8 ± 1	18.4 ± 5.4	5.5 ± 1.5
	35°	1.1 ± 0.4	7 ± 1	17.5 ± 6.1	3.8 ± 1.7
	40°	0.4 ± 0.1	6 ± 2	6.8 ± 2.1	9.4 ± 3.4
	45°	0.1 ± 0.03	5 ± 1	2.7 ± 1.1	2.6 ± 3.7
brown comb with silk	25°	4.3 ± 0.5	16 ± 4	29.9 ± 10	20 ± 4
	30°	4.4 ± 1.1	15 ± 1	30.5 ± 10.2	16 ± 4
	35°	4.6 ± 1.1	16 ± 3	29.6 ± 7.9	17 ± 5
	40°	3.0 ± 0.9	14 ± 3	22.9 ± 8.5	10 ±
	45°	2.1 ± 0.4	13 ± 2	16.7 ± 4.5	7 ±

The contribution of the silk fibre to the composite is also reflected in stiffness, a measure of the initial resistance of the material to deformation. A fall in stiffness with rising temperature was common to both white wax and the composite comb. However, when the composite was least tenacious it was comparable in stiffness to white comb wax when it was unyielding (Table 2). The energy of fracture for reinforced comb and white wax was similar in trend to that of stiffness. The very rapid rate of decline in the structured integrity of comb wax with rising temperature was thus greatly moderated by reinforcement with fibroin (Table 2).

CONCLUSIONS

The honeybee nest contains areas for the storage of nectar and pollen and those for the rearing of brood, either in the same or in different combs. On re-use over a number of brood cycles, brood comb becomes a composite material consisting of an elastic element, silk, embedded in a plastic one, wax; honey-comb tends to remain monophasic. The native silk of brood comb is a hygroscopic, anisotropic, crystalline fibroin whose dry form is structurally superior to its wet state. Dryness of the fibroin is achieved by its incorporation into the wax of the cell wall and possibly by the addition of a size. Anisotropy is exploited by cocoon-spinning larvae which play out the fibre to form sheets of random array. The strength and rigidity of silk is maintained between 25 and 45°C. Native wax is hydrophobic, isotropic and para-crystalline plastic whose strength and rigidity greatly decrease with increasing temperature. Propolis, often added to combs,

is mechanically similar to wax but structurally inferior to it. The elastic and plastic elements behave very differently with respect to temperature.

Intact brood comb is a planar isotropic silk-wax composite material in which the silk acts as a fibre reinforcement that greatly improves the overall mechanical properties of the comb. With increasing use, the percentage of silk present in the cell walls increases and so improves the load-bearing capacity of comb. The integrity of the composite is highly dependent on temperature. At 40°C, the wax phase is a viscous plastic that contributes virtually nothing to the functional composite so that the latter is compromised to the point of failure.

Brood comb, like many composites, is a compromise of the properties of two very different materials which, in combination, provide a structure with qualities that the individual constituents lack. In this instance, the mechanical properties of the composite are extremely sensitive to small changes in temperature that naturally occur. Inasmuch as honeybees die in droves at 40°C and the entire nest is likely to fail at this temperature, the survival of a colony of bees is predicated on thermoregulation. In view of the thermal tolerances operative on maker and material, it would appear that both evolved in a mutualism mediated by thermoregulation.

The compromise of bee and comb at the regulated nest temperature of about 35°C may be extended in another direction as well. What honeybees may have gained in improved material performance at a lower temperature, say 25°C, would have cost them minimally twice as much in expenditure on construction because of the temperature-dependent workability of their materials. Such increased costs would prevail irrespective of losses in the efficiency of energy production and its conversion into useful work.

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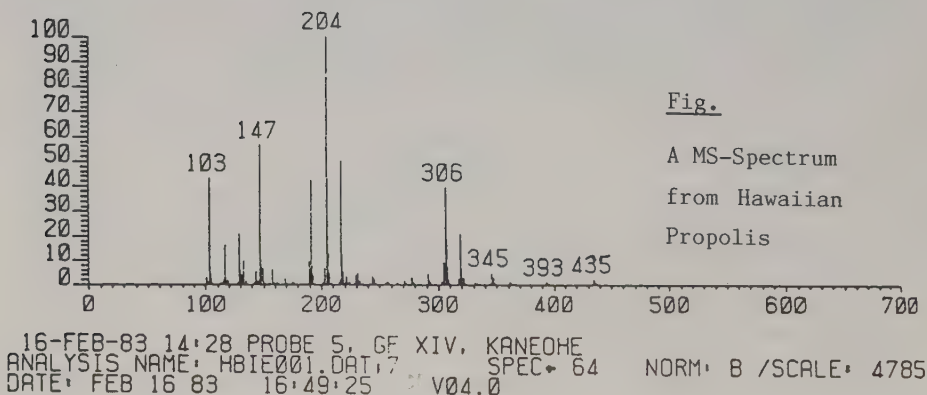
FREE COMMUNICATIONS

From which Sources do Social Bees gather Resinous Materials?

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Collection of tree resins by bees may have originated first for obtaining building materials, but may have been retained after evolution of wax production because of the antimicrobial and antiviral properties of many of the resins used, now known as propolis. A compilation of information upon propolis plant sources is given together with an outline of recent own chemical investigations of propolis samples of different geographical origin. In holarctic samples, e.g., dicaffeoylic compounds have been detected for the first time. Further, hawaiian samples show antiviral activities although they are lacking the caffeoylics, which cause antiviral activity of holarctic samples. On the other hand, they contain compounds with rather new types of both UV- and MS-spectra (see e.g. the fig. below).



However, because from many areas of the world nearly no information is available upon the plant sources of resins collected by bees, it is the primary aim of this contribution to draw attention to this particular field of apoid ecology and to encourage the congress participants to communicate observations, informations about publications in regionally confined journals and contacts to interested colleagues to the author.

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Quantitative Analysis of Variation in *Polistes* Nest architecture

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This poster presented information from a detailed analysis of the growth and architectural variation in nests of *Polistes annularis* in natural sites in Northeast Kansas, USA. Nest orientation is non-random: 62 nests in 1985 were oriented Northeastward. During the period of nest foundation the wind blew North on average. In a sample of nests pooled from 1984 and 1985, the number of cells in the nest before worker emergence was closely related to the number of foundresses ($N=16$, $y=41.5+25.4x$, $r=0.90$) and continued to be ($y=183.8+64.9x$, $r=0.76$, three weeks after worker emergence). The rate of construction of cells per day in 1985 was also closely correlated to foundress number ($N=14$, $y=1.0+0.4x$, $r=0.76$) and suggests an efficiency of about 30% for each additional foundress relative to a single queen working alone. Experimentally damaging nests increased construction efficiency for approximately a week, two of six colonies working as efficiently as lone queens.

A radial histogram technique for examining nest growth was presented and used to demonstrate that nests with the face oriented downward grew by random expansion while those oriented more horizontally grew asymmetrically downward. Nests also appear to grow more rapidly toward light. An analysis of 5 and 7 sided cells (1% and 0.1% of the total number of cells respectively) demonstrated that these anomalies occurred in worker comb, but very rarely in foundress built comb. Five sided cells were located below the midline of the nest while 7 sided cells appeared above the midline. Furthermore, 5 sided cells occurred at a relatively constant rate with respect to distance from the central, foundress built comb, but 7 sided cells occurred mostly at the boundary between foundress and worker built comb. Future work will include examination of the causes of curvature in the comb.

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2.1 Nutrition

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How far is Colony Development of Honeybees (*Apis mellifera*) influenced by Nitrogen, Amino Acids and Minerals from Pollen?

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Unsatisfactory performance and unexplained excessive losses of honeybee (*Apis mellifera*) colonies are commonly attributed to inadequate pollen supply. To test this hypothesis, we launched a field survey in 1978. Botanical and biochemical properties of the collected pollen, as well as population dynamics of free flying colonies were recorded.

The average yearly pollen harvest of 74 colonies at 5 apiaries was 18.9 kg (lowest/highest yields per apiary 11.6, 28.7; standard deviation within apiaries 6.5). Pollen harvest was positively correlated with number of broodcells raised ($r = 0.60$) but only weakly with population peak, i.e. maximum number of workerbees ($r = 0.27$). There is a negative correlation between average longevity and the amount of pollen collected per bee and per day ($r = -0.40$). Computations of nitrogen balance demonstrate that some colonies use pollen very efficiently (utilization ratio > 50 %). Within years however, the rhythm of brood production is largely independent of pollen supply. Even early in spring and at the end of June when pollen abundance is insufficient, no throttling of brood was observed. Neither body weight nor nitrogen content of adult worker bees are much affected by excessive or insufficient pollen harvest. In May, July and August, only part of the pollen collected is converted into brood, the rest is stocked or eliminated. In addition to nitrogen, some amino acids and minerals supplied by pollen were studied in a similar manner.

Our results concern exclusively free flying colonies. They often do not coincide with those of other authors who worked with confined bees in cages or flight rooms. They are only valid under the condition that a minimal amount of 10 - 11 kg pollen per year and colony is harvested. Though no direct link between pollen supply and colony breakdown could be demonstrated, the survey yielded much detailed new information about the biology and ecology of honeybee colonies in Switzerland.

In the following diagram the methods of investigations are shown. The numbers refer to the bibliography and the posters presented at this congress.

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SYMPOSIUM

Bees, Protein and Amino Acids

Organizer: Hans Wille

Introduction

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Pollen and nectar/honeydew are essential for the bee colony. Pollen is the source of proteins and minerals, nectar and honeydew of carbohydrates. By feeding sugar syrup, the beekeeper can help the colony to overcome periods of lack of nectar or honeydew. Attempts to compensate pollen scarcity by feeding a proteinaceous substitute, however, were not very convincing so far.

The first group of topics of the symposium focusses on "ecological aspects". The questions are "what", "when", "where", "how" and "in which quantities" do the colonies get their basic nutrients in nature. An investigation on honeybees in Liebefeld (Central Switzerland), covering several years, shows that there are only a few pollen species which provide the bulk of the annual harvest. The Meliponide *Plebeia saiqui* in Brazil is similar in respect to harvesting behavior. The chemical composition of the 100 most important pollen types was analyzed. Water content, nitrogen, amino acids and minerals such as Na, Ca, K, Mg and P were measured. Hence it is possible to compute for each constituent, how much was collected by a particular colony at a particular time.

In the second and third group of contributions, "Nourishment of *Apis mellifera*" and "Protein metabolism", questions are risen about how the colonies dispose of their nutrition. Balance sheets were drawn for nitrogen metabolism by comparing the amounts bees add to the colony by harvesting pollen with the amounts incorporated into the brood and lost with disappearing bees. The protein content of summerbees and winterbees was determined as a function of their age. Sugars and proteins were studied in different larval jellies. No significant correlation could be drawn between colony development, weather conditions and differences in quality of larval jellies. The nitrogenous components and carboxylic acids of royal jelly were subjected to detailed analyses. Protein synthesis of juvenile hormone dependent systems was studied as well as changes and metabolism of hemolymph protein patterns in larval and worker honeybees as a function of age and physiological condition.

There is now an almost general acceptance of "nutritional conditioning" as the basic requirement for queen differentiation. To test this concept, the composition of larval food of seven species of stingless bees was analyzed. The free amino acid composition is very variable during larval development while the polypeptide patterns of larval food shows a close relationship to evolutionary aspects of stingless bee phylogeny.

The fate of glucose ingested as an aqueous solution by winterbees was studied. Only a small proportion reaches the rectum. The main part enters the hemolymph through the epithelium in the anterior two thirds of the midgut.

Since World War II, the industrialized countries have seen important changes in their environment resulting in a shifting in available nutrients to bees. The effect on pollen was a reduced number of plant species offering bulk supply. What is the effect of this change in pollen supply on colony development? As long as a minimum quantity of pollen is provided, bee colonies behave as an astonishingly well buffered social system. They are able to adapt to the new situation and to the sometimes very scarce pollen supply in a most efficient way.

Exploitation of Floral Resources by *Plebeia saiqui* Friese (*Apidae*, *Meliponinae*)

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Plebeia saiqui is a small stingless bee (4 mm) forming medium populated colonies of ca. 7000 individuals. As is the case in some other species of this genus, flight activity is strongly affected by abiotic factors, in particular by temperature (Imperatriz-Fonseca et al., 1985). These bees have a rather inefficient communication system for food sources (Lindauer & Kerr, 1960). They are opportunistic foragers, timid and non-aggressive (Johnson & Hubbell, 1974). Such bionomic features could limit home range and foraging periods and might influence the pattern of utilization of floral resources. Here we evaluate the exploitation of floral resources by *P. saiqui*. We calculated some ecological parameters and related them to the bionomics of this species and to the floral structure of the study site.

MATERIALS AND METHODS

The colonies of *P. saiqui* were located in the gardens of the Bio-sciences Institute at Sao Paulo University Campus (IBUSP), on the outskirts of Sao Paulo city (23° 33'S; 46° 43'W). In these gardens there is a diversified native and foreign flora and a small semideciduous forest covering 10 ha. During 1981, pollen and honey samples were taken from two colonies of *P. saiqui* (A and B) and acetolysed according to Erdtman (1960) and Iwana & Melhem (1979). The frequency of pollen types in the samples was estimated by counting 1000 grains (Vergeron, 1964) on three slides. These frequencies were used to calculate niche width - $H' = -\sum p_i \ln p_i$ (Shannon-Weaver, 1949); evenness - $J' = H' / \ln N$ (Pielou, 1977), and percentage of similarity - $PS = 1 - 1/2 \sum |p_i - q_i|$ (Schoener, 1968), Where: p_i = the pollen proportion of the plant species in each sample; N = total number of pollen types in samples.

RESULTS AND DISCUSSION

We found a large number of pollen types in honey and pollen samples (Fig. 1A) and determined 164 plant species belonging to 52 families. In general these data concur with the average correlation between individual and colony population size, home range, period of foraging activity and diversification of harvest as described for *T. spinipes* and *A. mellifera* (Cortopassi-Laurino & Ramalho, 1986)). The average values of niche width and evenness (Table 1) reflect the high monthly variation in harvest, with intensive exploitation of just a few floral sources. Other species showed a similar pattern of harvest diversity in this bee-plant community (Cortopassi-Laurino & Ramalho, 1986).

Table 1 --	NICHE WIDTH (H')		EVENESS (J')	
	Pollen	Honey	Pollen	Honey
Col. A	1.29	1.20	0.53	0.47
Col. B	1.09	1.29	0.41	0.41

The plant families represented in the samples with greater numbers of species were also frequent in the gardens of IBUSP (Fig. 1A). Some of the most intensively visited plant families (Fig. 1B) are poorly represented in respect to the number of species growing in the gardens. Examples

being Balsaminaceae, Moraceae and Palmae. Thus the distribution of foragers at food sources is probably coincidental, but there is preferential utilization of some plant taxa.

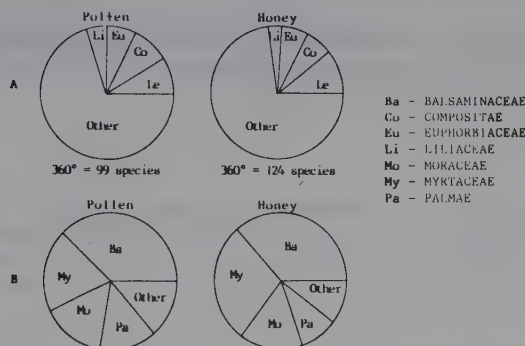


Fig. 1 -- Pie diagram of the most represented plant families in honey and pollen samples. A- in number of species; B- in frequency.

On average the high values of PS for pollen (59.51) and honey (66.22) reveal a similar proportional use of some resources by both colonies. *Impatiens sultanii* (Balsaminaceae), *Cecropia* sp (Moraceae), *Eucalyptus* spp (Myrtaceae) and *Archontophoenix* (Palmae) were the most conspicuous examples. Particularly *I. sultanii* is commonly found as part of the diet of other species of *Plebeia* (Imperatriz-Fonseca et al., 1986). We believe that these data reveal floral preferences by *P. saiqui* and use of some resources which this species does not share with other bee species, with higher colonial necessities such as *T. spinipes* and *A. mellifera* (Cortopassi-Laurino & Ramalho, 1986).

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The Pollen Types Collected by Honeybees (*Apis mellifera*) in Switzerland

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The yearly pollen harvest of *Apis mellifera* colonies at 5 apiaries in different parts of Switzerland was surveyed from 1980 till 1984. Calibrated pollen traps were installed for one day per week. The trapped pollen was identified microscopically. The total amount of pollen collected by the colony was calculated by extrapolation based on the previously verified trap efficiency and on meteorological data. More than 320 different pollen types could be identified. The number of pollen types per colony and year varied from 60 to 120. Each apiary exhibits its own distinct pollen pattern. There is, however, important variability from year to year and from colony to colony. Cultivated plants such as *Brassica* sp, *Zea mays*, *Trifolium repens* and sometimes orchard trees are among the most important pollen suppliers. Pollen of anemophilous plants was found in considerable amounts. Frequently, 90% of the yearly harvest of a colony is composed of fewer than 25 different pollen types.

THE 10 MOST IMPORTANT POLLENTYPES AT THE FIVE APIARIES
g pollen (air dried) per average colony and year

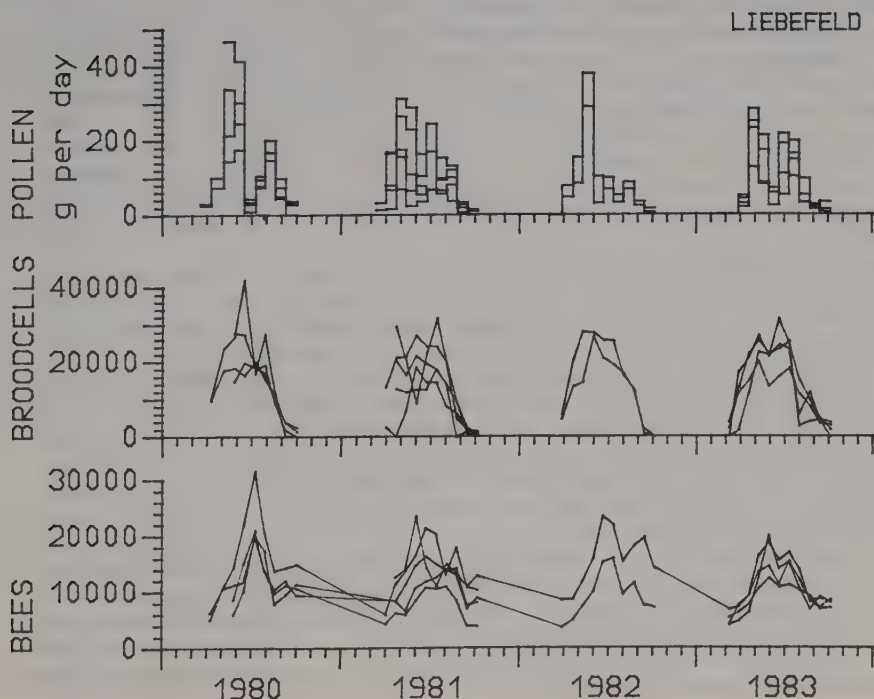
APIARY	LIEBEFELD	AATHAL	GALMIZ	S'NRIED	INTRAGNA
	CENTRAL	CENTRAL	CENTRAL	BERNESE	SOUTHERN
	PLATEAU	PLATEAU	PLATEAU	OBERLAND	SWZ'LAND
N OF COLONIES	15	15	13	18	14
1 <i>Brassica</i> sp (rape)	1893	4056	5948	62	-
2 <i>Trifolium repens</i>	984	1506	4955	521	7
3 <i>Zea mays</i>	2051	4684	190	-	-
4 <i>Acer</i> sp	2542	179	1526	1301	736
5 <i>Taraxacum officinale</i>	528	1086	1461	1785	439
6 <i>Trifolium pratense</i>	381	87	2023	2036	-
7 <i>Salix</i> sp	995	336	2026	148	544
8 <i>Plantago lanceolata</i>	979	1681	194	193	54
9 <i>Castanea sativa</i>	2	-	-	-	3125
10 Kernel fruits	535	450	21	178	1405
OTHERS	8881	6108	10054	5532	8113
TOTAL	19770	20174	28397	11755	14424

Pollen Harvest and Colony Development of *Apis mellifera* in Switzerland

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Honeybees, like all Apoidea, depend on pollen from flowering plants for proper nutrition. Amino acids, proteins and most essential mineral elements found in honeydew and nectar are by far insufficient to cover the needs of a colony. Pollen is the most important link between plants and bees. The economically oriented beekeeper commonly attributes poor performance of the colonies, diseases and other malfunctions to poor or unbalanced pollen supply.

Pollen harvest and colony development was investigated 1980 - 1984 at five apiaries in Switzerland. Bee density in Switzerland is one of the highest in the world and geographic and vegetational conditions are very diversified. Seventy-four free flying colonies were studied at Liebefeld, Aathal and Galmiz (Swiss central plateau), Schönried (Bernese Oberland) and in Intragna (southern Switzerland, Ticino). Colony dynamics were recorded by measuring brood and number of adult bees every three weeks. Pollen harvesting activity was recorded by using calibrated pollen traps one day per week and the total amount of pollen collected was calculated by extrapolation using meteorological data.



AVERAGES		LIEBEFELD				FIVE APIARIES
		1980	1981	1982	1983	
	number of colonies	4	5	2	4	74
P	Pollen, kg dry weight	21.2	18.1	17.7	18.0	17.9
PN	Pollen, g nitrogen	756.8	620.5	622.1	672.3	651.3
BR	thousand broodcells	117.9	113.5	144.4	136.4	121.8
BD	thousand bee days	2215	2224	2463	2232	2448
P/BD	mg pollen per bee day	10	8	7	8	7
P/BR	mg per broodcell	187	162	123	131	148
PN/BD	microgram N per bee day	356	277	262	298	265
PN/BR	mg N per broodcell	6.6	5.5	4.3	4.8	5.3
PN/P	mg N per g pollen	3.5	3.4	3.4	3.7	3.8
BD/BR	bee days per broodcell	18.8	19.7	16.7	16.4	21.0

CORRELATIONS 74 colonies, critical $r(0.01) = 0.302$; $r(0.001) = 0.380$

	P	PN	BR	BD	P/BD	P/BR	PN/BD	PN/BR	PN/P
P	1.000								
PN	0.989	1.000							
BR	0.602	0.624	1.000						
BD	0.443	0.457	0.614	1.000					
P/BD	0.876	0.857	0.355	-0.007	1.000				
P/BR	0.732	0.697	-0.048	0.049	0.800	1.000			
PN/BD	0.882	0.889	0.402	0.039	0.983	0.762	1.000		
PN/BR	0.772	0.765	0.020	0.104	0.818	0.977	0.817	1.000	
PN/P	0.182	0.313	0.320	0.302	0.069	-0.041	0.239	0.153	1.000
BD/BR	-0.296	-0.305	-0.635	0.160	0.396	0.179	-0.408	0.136	-0.097

Brood rearing resumes in February or March. Substantial pollen harvest begins only in April. A pronounced increase of the number of adult bees, however, is observed only in May. Insufficient pollen harvest early in the year or during midsummer is not necessarily reflected in brood rearing. The number of bees is not closely related to breeding. This indicates that the bee colony as a system is buffered. Pollen is not immediately converted into brood: it is stored, lost, varies in turnover and conversion rate. For the bees, the rate of emergence changes and the duration of life of adult bees is highly variable.

Variations from colony to colony, from year to year and from apiary to apiary demonstrate the effects of location and, to a lesser degree, the annual changes in weather and vegetation. The variation due to individual traits of honeybee colonies, however, is very important. The amount of pollen harvested from April to September is positively correlated to almost every parameter studied, except to the longevity of the workerbees (bee days/broodcells) and to average nitrogen content of the pollen. Most of the significant correlations, however, explain only a minor part of the variation. This means that pollen harvest, brood rearing and colony strength interrelate in a complex multifactorial system.

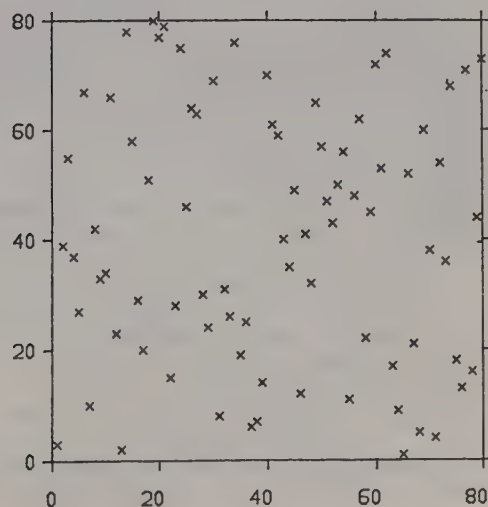
At apiaries in central Switzerland, pollen supply is based on bulk pollen suppliers such as rape, maize, some grasses and clover. The colonies in general do not suffer from pollen shortage, mainly because of the effects of modern agriculture. Pollen, although crucial for proper nutrition, is not the only factor for the regulation of colony development. Its impact is indirect.

Amino Acids in Pollen Collected by Honeybees

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Pollen collected by *Apis mellifera* colonies in different apiaries of Switzerland were analyzed in order to determine their nutritional potential. Ninety-nine samples of the major pollen types were hydrolyzed in hydrochloric acid and their amino acid composition determined. The total amino acid content varied from 5% of dry weight for *Pinus* sp. and *Cupressaceae*, to 33% for *Sarothamnus scoparius*. In general, *Leguminosae* pollen were richer in amino acids, *Gymnospermae* and *Monocotyledonae*, as well as pollen from other anemophilous plants were poorer. Apart from these differences, however, amino acid content is not a good indicator for the systematic position of a plant. Amino acid composition does not vary much between pollen types. Except for histidine, arginine, and methionine, there is a very close correlation between the levels of individual amino acids and the total amino acid content. The fraction of essential amino acids amounts to 44% on average. With pollen supply of mixed origin there is hardly a risk of unbalanced nutrition with respect to amino acids. If the honeybees were able to focus on harvesting selected pollen species, they could substantially improve the nutritional value of their pollen yield. There was no correlation, however, between the amount of a pollen type gathered per year and its amino acid content. We therefore conclude that *Apis mellifera* are directed by aspects other than amino acid content, in selecting their pollen plants.

Figure:
Ranks of 80 pollen
species collected by
Liebefeld colonies in
1980 and 1981, amount
(abscissa) vs amino
acid content
(ordinate).



Nitrogen Components and Carboxylic Acids of Royal Jelly

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The royal jelly (RJ) is a secretion from the hypopharyngeal gland (HG) and mandibular gland of the 3-12 days old bee (*Apis mellifera* L.) (Shinoda and Chuto, 1978). The RJ has been well known as a valuable food source for honey bee larvae by dint of its nutritional and physiological properties. Whether the bipotent female larva develops into a adult queen or a worker, depends on the quality of RJ supplied during a critical development phase (Weiss, 1978). In this respect, the importance of RJ was emphasized. The work reported herein deals with the chemical components of RJ in relation to the nutrition of honey bees.

MATERIALS AND METHODS

Nitrogen compounds were separated by column chromatography and homogeneity of proteins was determined by isoelectric focussing, polyacrylamide gel electrophoresis, ultra centrifugation and immunological methods. Carboxylic acids were separated by GC and HPLC. The moisture content was determined in a vacuum oven at 60°C. Nitrogen (N) was determined by micro Kjeldahl. Ether extract was analyzed by Soxhlet extraction, ash was analyzed by muffle furnace at 550°C and various minerals were determined by atomic absorbance photometer. Total sugar content was determined by Somogyi method and was calculated in terms of total reducing substance as glucose. The acidity was determined by alkali titration using 0.05N NaOH. Glucose oxidase activity was analyzed by modification of the method of Schepartz (Schepartz, 1964). Antibiotic activity was determined by paper disc method.

RESULTS AND DISCUSSION

The chemical composition of 18 samples of RJ were analyzed. The average value of moisture of fresh RJ was 66.8%, crude protein 12.13%, total sugar 11.51%, ether extract 5.1%, ash 1.0%, unknown 3.1%, total acidity 44.0 ml (ml 1N NaOH/100 g RJ) and pH 3.8. Moisture, crude protein, sugar and acidity of RJ varied according to the ages of larvae, but ether extract and ash content did not. The total nitrogen content made up 2.0% on fresh RJ. Most of water soluble N was proteinous N (Table 1). Most of the proteins in whole RJ were in the range of about

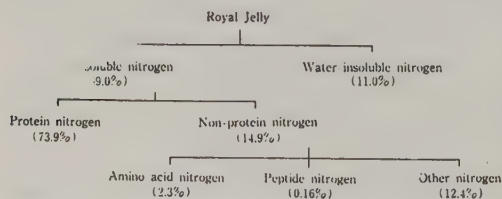


Table 1. Nitrogen distribution of RJ

32.5Kd to 87.6Kd. The 53.3Kd protein was predominant. The protein pattern was also essentially the same in RJ and HG homogenate of 7 day old bees, indicating that most RJ proteins were biosynthesized by HG during the nurse bee stage. Only a 78.9 Kd protein was not present in HG homogenate of 7 day old bees.

The four major proteins were separated from RJ and all four proteins were glycoproteins containing glucose and mannose. There was a significant difference of amino acid composition between these proteins.

The glucose oxidase usually found in HG of flight bees was active in the water-soluble protein fraction of RJ, but its activity was very low.

Five peptides were isolated from the non-protein N fraction. Their amino acid composition differed from each other, their molecular weight was, however, about 1.6Kd.

The twenty nine kinds of free amino acids and their derivatives were identified in RJ. The amount of free proline was 58% of the total free amino acid content and the next highest level was 15% lysine. The pattern of free amino acids of RJ was similar to the hemolymph of 10 day old workers and 10 day old queens, but taurine and β -alanine found in RJ were not present in those of the worker bee (wand. 1970). γ -Aminobutyric acid, L-methylhistidine were not present in those of the queen bee.

The thirteen carboxylic acids were identified from RJ (Table 2).

Carboxylic acid	Chemical formula	Amount (mg/fresh RJ100g)	Relative amount (%)
OcA	$H_3C(CH_3)COOH$	3	0.1
3-HOcA	$H_3C(CH_3)_2CHOHCH_2COOH$	16	0.3
OcDiA	$HOOC(CH_2)_2COOH$	24	0.4
8-HOcA	$HOCH_2(CH_2)_6COOH$, $HO-C_6H_4-COOH$	328	5.6
p-HBA	$HO-C_6H_4-COOH$		
3-HDaA	$H_3C(CH_3)_2CHOHCH_2COOH$	108	1.9
10-HDaA	$HOCH_2(CH_2)_8COOH$	1254	21.6
10-HDeA	$HOCH_2(CH_2)_8CH=CHCOOH$	1847	31.8
DaDiA	$HOOC(CH_2)_4COOH$	83	1.4
2-DeDiA	$HOOC(CH_2)_2CH=CHCOOH$	158	2.7
3, 10-HdaA	$HOCH_2(CH_2)_8CHOHCH_2COOH$	104	1.8
Glu A (including Glu L)	$HOCH_2(CHOH)_4COOH$	1394	24.0
Unknown		491	8.4
Total		5810	100.0

Table 2. Chemical composition of carboxylic acids in RJ

The ether soluble hydroxylic acid possessing a chain length of C₈ and C₁₀ accounted for 63% and the ether soluble dicarboxylic acid for 5% of the total respectively. The major components of the carboxylic acids in the ether-soluble fraction were 10-hydroxy- Δ^2 -decanoic acid and 10-hydroxydecanoic acid, in the water soluble fraction gluconic acid. Furthermore, 3-hydroxyocta-

noic acid was identified for the first time. The bacteriostatic activity of 10-hydroxy- Δ^2 -decanoic acid was greater than that of the other

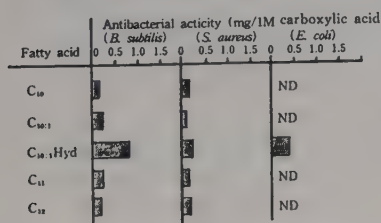


Fig. 1. Bacteriostatic activity

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Sugar and Protein in the Food for Honeybee Worker Larvae

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The composition of royal jelly is well known. This study concerned dry weights, sugars and amounts of protein in jelly fed to worker larvae at an age of 2-3 days (young larvae) and of 4-5 days (old larvae) during nearly the whole breeding season. Weight and protein content of larvae were also investigated. Additionally, population dynamics and availability of food outside the hive were recorded.

MATERIAL AND METHODS

In 1983 and 1984 two hives with 10 to 20 frames were used simultaneously in the south of Styria. Their queens were caged on a brood comb for one day. Six and eight days after egg-laying combs were removed from the hives and samples of jelly and larvae were collected. Single samples were investigated from 1983, pools from 1984. Protein was analyzed with the Biuret and the Folin reagent, glucose, fructose and sucrose (sugars) with enzymatic kits (Boeringer Mannheim).

RESULTS

Dry weight, amount of protein and sugars in jellies from hives investigated in parallel were similar in most cases. Dry weight of food for young larvae ranged from 200 to 320 ($\bar{x}=262\pm5$) $\mu\text{g}/\mu\text{l}$, that for old larvae between 370 and 600 ($\bar{x}=458\pm10$) $\mu\text{g}/\mu\text{l}$. Water and dry weight of protein in food for young larvae was positively correlated ($p<0.002$), as were water and dry weight of sugar ($p<0.01$). During the period of investigation there were significant rising and decreasing tendencies in content of sugars and protein that could not be clearly correlated to population dynamics, to weather or to availability of food sources (results shown for 1984 in Figure 1).

The protein content of larvae was significant negatively correlated to their weight, the decrease of the curve in very steep for small, that means young larvae (Figure 2). With the method used no influence of larval food upon the protein content of larvae could be shown; the dispersions within the larval weights were too large.

Within a healthy colony there is a variance of quality of worker jelly but the differences seem to be within certain limits.

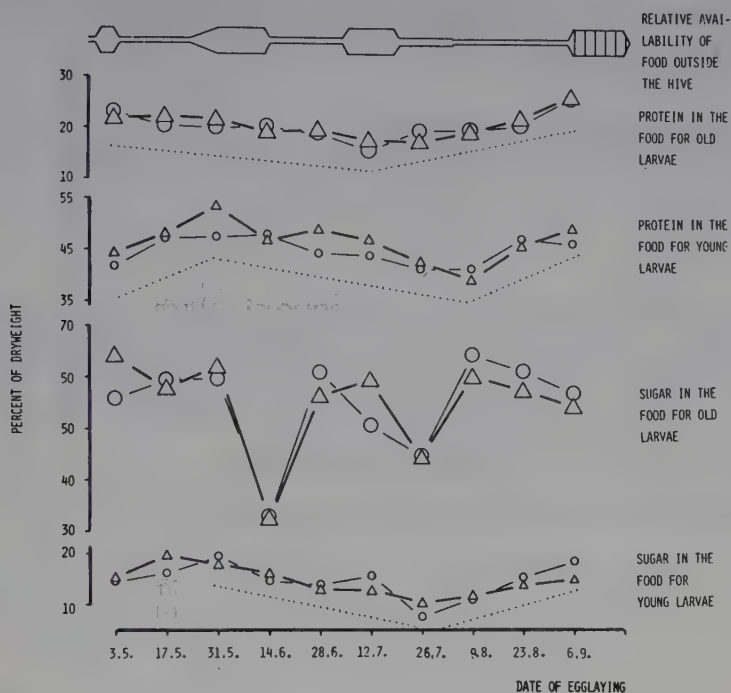


Fig. 1. -- Content of protein and sugar in the food for worker larvae in dependence of season (1984). Each symbol represents the square of two pools consisting of 25 samples each. Significances of de- or increasing tendencies are calculated with values from hive 1 (o) and 2 (Δ) and are indicated by dotted lines. Thickness of topmost graphs shows relative availability of food outside the hive, striped segment indicates winter feeding.

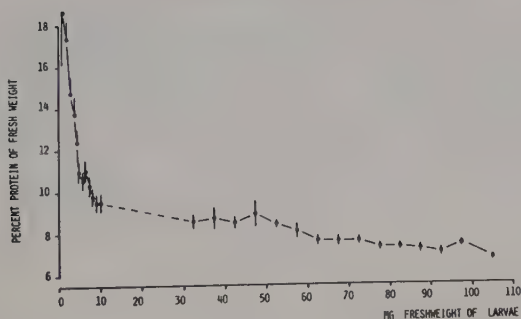


Fig. 2. -- Percent of protein from fresh weight of larvae in dependence on fresh weight (1983). Indicated are means and standard error of mean ($n=5-22$).

Protein Synthesis by Hypopharyngeal Glands of Worker Honey Bees

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During the nursing phase, the hypopharyngeal glands (HG) of worker honey bees (*Apis mellifera* L.) are fully developed in size and secrete an essential proteinaceous part of the larval food. Later, when the workers become foragers, the hypopharyngeal glands decrease in size and, in a second phase of glandular function, produce a number of enzymes, including the honey digestive sucrose (Halberstadt, 1980).

In order to study this synthetic activity during the lifespan of adult worker honey bees, the HG were cultured *in vitro*, and the incorporation of radiolabeled amino acids into protein was analyzed.

MATERIALS AND METHODS

Worker honey bees were reared during early summer in strong colonies, marked at emergence, reintroduced into the same colony and removed for examination at various stages during their adult life cycle.

The HG were dissected and cultured in a medium originally developed for honey bee fat body and ovary (Kaatz et al., 1985). This defined medium supported 18-fold higher rates of protein synthesis and better viability than a medium previously used for culturing HG *in vitro* (Brouwers, 1982). In our medium the glands survive for at least 24 hours.

Protein synthesis of HG was analyzed after one hour of incubation in radiolabeled medium (^3H -leucine, 25 $\mu\text{Ci/ml}$). Four HG were cultured together. After homogenization of the HG by sonification, label incorporation into water-soluble proteins was measured by TCA precipitation (10%) on filter paper discs. The synthesis of larval food proteins was analyzed by indirect immunoprecipitation of HG-homogenates with an antiserum raised against Royal Jelly (RJ) and Protein A. The HG polypeptide pattern was examined by SDS-PAGE, the pattern of synthesis by fluorography.

RESULTS AND DISCUSSION

Rates of protein synthesis in hypopharyngeal glands vary in accordance with age and physiological status of the worker honey bees (Fig.). Protein synthesis in HG starts between imaginal eclosion and emergence from the brood cell. During the first four days of adult life the synthetic activity of the HG greatly increases. On day 4 the proportion of RJ proteins amounts to 90% of the total protein production.

During the nurse bee stage and especially between day 10 and 14, the glands synthesize proteins at the highest rates observed (Fig.). In this phase they mainly produce RJ proteins. Between day 4 and 17 the HG contain predominant polypeptides of molecular weights of 48, 54, 64 and 69 kd. Their molecular weights are identical with those of the major larval food polypeptides, indicating the storage of such products in the HG before secretion. Especially the polypeptides of 48 and 54 kd are produced at high rates since they are predominantly labeled in the fluorograms.

After day 14 the synthetic activity of the HG rapidly declines by 36% and remains stable between day 17 and 30. During this period, the synthesis

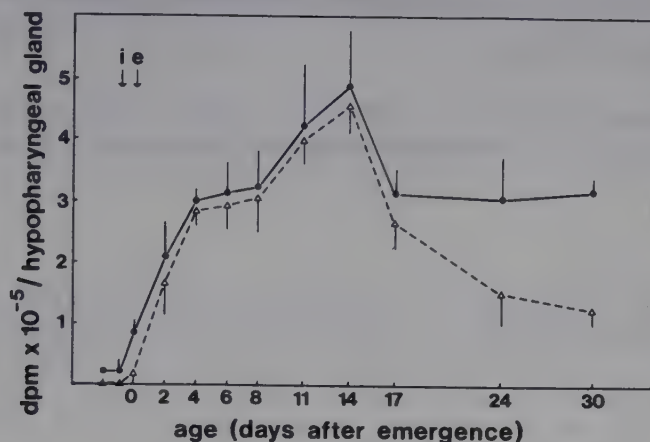


Fig. Rates of protein synthesis of hypopharyngeal glands during the lifespan of adult worker honey bees. In vitro tracer incorporation into total water-soluble HG proteins (●) and Royal Jelly proteins (Δ). Mean values and s.d. of 5 determinations. i=imaginal moult, e=emergence from the brood cell

of RJ proteins decreases over-proportionally, but surprisingly never drops to zero level. Even in 30 day old bees - which were identified as active foragers by their pollen loads - the synthesis of RJ proteins accounts for 45% of the total protein production. At this age the HG contain only small amounts of protein. But the glands are still actively synthesizing proteins, some of these being strongly labeled: The RJ polypeptides of 48 and 54 kd, and other major, field bee-specific ones of 89, 78 and 70 kd. However, the low protein content in the glands indicates that the newly synthesized proteins have not been stored.

Halberstadt (1980) postulated two phases of hypopharyngeal gland activity: a first period of larval food production and, after a switch in the synthesis program during the transition from nurse to field bee, a second one of enzyme production. In contrast, we observed - by looking directly at the pattern of synthesis - a presumably functional differentiation: the production of new field bee-specific proteins begins without a loss of capability to produce larval food proteins. Larval food proteins seem to only be synthesized at different rates in an age-dependent pattern during the entire imaginal life of worker honey bees. This enables the bees to rapidly adapt themselves to colonial conditions and to perform whatever task is necessary in the hive. It remains unresolved whether workers regulate the larval food composition primarily by selective secretory mechanisms as is partly evident by our data or rather by differential gene activity for secretory proteins. This will be studied in the future.

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Movement of Glucose Solution in the Midgut of Honeybees in Winter

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During winter months in Austria honey bees (*Apis mellifica carnica* Pollm.) must perform much metabolic work to heat their hive and have less chances to fly and to empty their rectum. Transport of liquid food from the crop into the midgut of bees in winter is regulated by the proventriculus and depends on concentration of the food (CRAILSHEIM, 1985).

MATERIAL AND METHODS

Glucose solution was traced by ^3H -glucose and ^{14}C -polyethylene glycol, the latter not penetrating the gut wall and being absorbed only weakly by gut material. Bees with empty crops were fed 10 μl 0.5-3.0 M glucose solution and kept for 30 minutes in cylinders (volume 5 ml) at 25°C. A second group was handled identically but not fed. Afterwards midguts were dissected, weighed and investigated for ^3H and ^{14}C either in total or cut into three parts of about identical length.

RESULTS

During 30 minutes about 9 μl of the 10 μl 1M glucose fed had passed from the crop into the midgut. The weight difference of midguts of fed and non-fed bees is about 2.5 mg at the end of the 30 minutes. Most of the 9 μl have disappeared. Because polyethylene glycol cannot penetrate the gut wall and is found nearly quantitatively within the midgut, liquid could not have been transported backwards to the rectum in significant amounts. It must have gone into the haemolymph. If glucose solutions of 0.5 - 3M are fed and the midgut is dissected into three parts after 30 minutes, at least 97% of the polyethylene glycol that had come into the midgut can be found in the first two-thirds. At most, 24% of the ^3H of glucose can still be detected in that region and much less in the following third.

It can be concluded from these results that only small amounts of liquid reach the rectum passing the midgut and that most of the glucose penetrates the gut wall in the first two-thirds of the midgut.

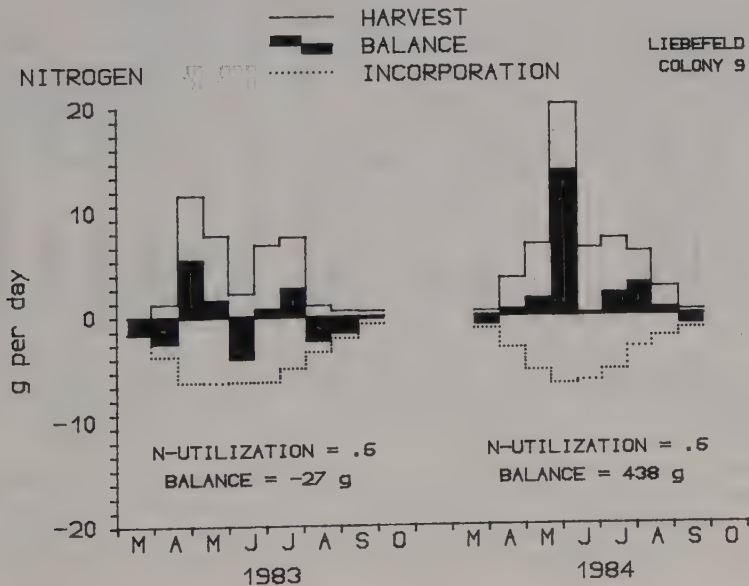
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The Nitrogen Balance of Free Flying *Apis mellifera* Colonies

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Nitrogen content of larvae, of adult honey bees (at intervals of 10 or 11 days) and of bee-collected pollen from 4 colonies were observed at Liebefeld from March to October in 1983 and 1984 using the Kjeldahl method. The number of larvae and adult bees was recorded every three weeks in order to study population dynamics. Pollen was collected by calibrated pollen traps during one day per week and the total amount of pollen collected was calculated by extrapolation. The nitrogen balance was computed by comparing pollen harvest with incorporation into body biomass. There were no significant differences of nitrogen content of larvae between colonies or between years. Nitrogen content of adult bees was different from colony to colony but not from year to year. These differences however were without effect on population dynamics. Within colonies, changes of nitrogen content of brood and of adult bees during the year were not very important, and never reflected the marked fluctuations observed in pollen collection. In spring, and occasionally in June and September, pollen harvest does not cover for the net nitrogen need of the colony. Therefore, reserves must have been mobilized. It is concluded that honeybees colonies must convert more than 50% of the collected pollen nitrogen into body biomass to account for their net nitrogen balance.



Age Dependence of Fat Body Protein in Summer and Winter Bees (*Apis mellifera*)

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In worker bees seasonal, age-dependent changes of the pharyngeal gland, haemolymph titres of juvenile hormone, protein and vitellogenin have been reported (1). Also, fat body macromolecules (lipids, glycogen and protein) show a season dependent change (2). We conducted the present experiments in order to study the age- and season dependence of the fat body protein and the pharyngeal gland weight.

MATERIALS AND METHODS

The fat body of single worker bees of definite age were homogenized with a sonifier. After short centrifugation, the soluble protein in the supernatant was determined by the Bio-Rad method and its pattern was analyzed with gel permeation HPLC. The approximate molecular weight (MW) was determined by comparison with the separation pattern of proteins with definite molecular weight. The dry weight of the pharyngeal gland was determined after (1).

RESULTS

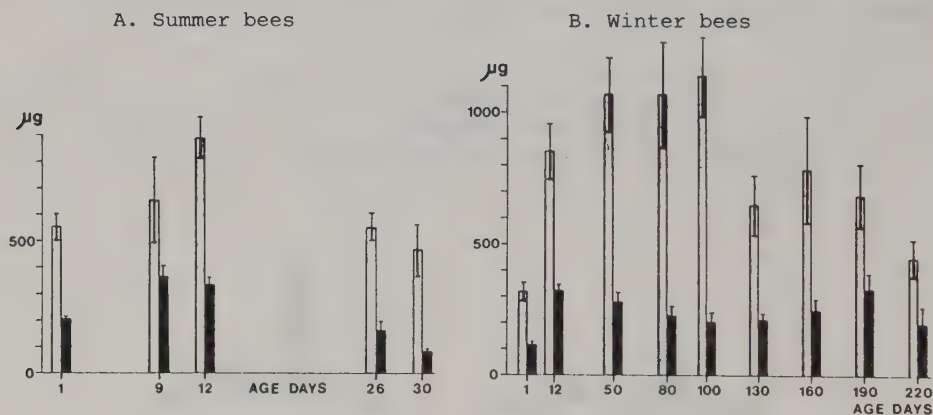
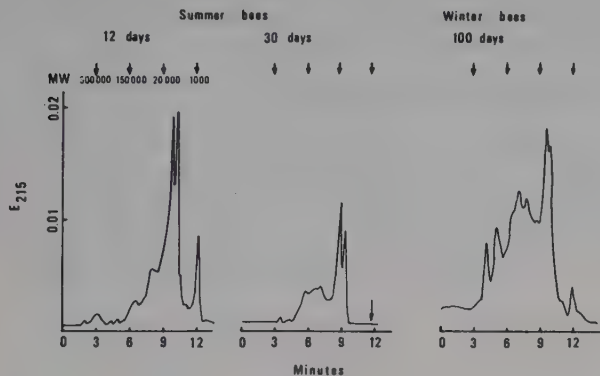


Fig. 1. -- Age- and season dependence of the weight of the pharyngeal gland (black columns) and fat body protein in worker bees (white columns).

In summer bees the protein content and the weight of the pharyngeal gland have a similar age dependence: there is a minimum in 1 day old bees, an increase to a maximum in 9-12 days old bees and a decrease to a minimum in 26-30 days old bees. Correlation of pharyngeal gland

weight to protein concentration gave a correlation coefficient (r) of 0.552 and $p < 0.001$ ($n = 240$).

In winter bees the age dependence pattern of the two parameters is quite different. The weight of the pharyngeal gland is small in 1 day old bees, increases in 12-50 days old ones and drops slightly in 80-130 days old bees. Then there is a new increase (when bees begin raising new brood) and a new decrease, when workers start foraging. The fat body protein is minimal in 1 day old bees, then increases to reach a maximum in 50-100 days old bees and after a short drop increases again somewhat, to reach a minimum in 220 days old bees - similar to the course of the pharyngeal gland. The correlation between protein and pharyngeal gland weight is highly significant, but less pronounced than in the summer bees: $r = 0.302$, $p < 0.001$ ($n = 210$).



Size exclusion chromatography with a Micropack 3000 Sw column (Varian) with 0.1 M Na-phosphate pH 7 buffer as solvent. Flow: 1.2 ml/min

Fig. 2. -- HPLC of fat body proteins

In 30 days old summer bees the protein concentration was half as much as in 12 days old bees and a protein with MW of 1000 was lacking. In 100 days old winter bees four new peaks with MW of 350,000, 220,000, 60,000 and 30,000 appeared and the peak with MW of 1000 was only about 10% of that in 12 days old summer bees. Vitellogenin is known to have a MW of 350,000 to 500,000. Its concentration in the bee haemolymph is higher in winter bees than in summer bees (1).

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Caseinolytic Activity in the Midgut of the Honeybee (*Apis mellifica* L.) in Dependence on Age and Season

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Changes in activity and function of worker honey bees (*Apis mellifica* L.) are also connected with changes in protein metabolism. Grogan and Hunt (1980) reported that the proteolytic levels in the midgut of house bees are higher than in forager bees. It was the aim of this investigation to study these changes more extensively on worker bees of known age and known function in the bee hive.

MATERIAL and METHODS

Worker honey bees were taken freshly hatched and 16-24 hours old from a honeycomb in an incubator or from an observation hive at ages of 3, 8, (14 or) 20 and 30 days. Parallel to the experiments on enzymatic activity, the condition of the hive, the begin of foraging of the investigated bees and the size of their hypopharyngeal glands were determined.

The midgut tissue and contents were separated, sonified in Tris-HCl at pH 8.2 (4°C) and centrifuged at 20000g for 20 min. Proteolytic activity of the supernatant was determined by degradation of casein (1% in Tris-HCl at pH 8.5 at 30°C). Reaction was stopped by addition of 10% TCA. The amounts of deliberated peptides and amino acids were determined with the folinreagent and given in equivalent of tyrosin.

From an aliquot of midgut contents, pollen was removed by centrifugation and soluble protein was measured according to Lowry et al. (1951) after hot alcoholic precipitation.

RESULTS

Most of the total caseinolytic activity can be found in the midgut contents, only 5.8 - 11.3% (varying in the different age groups) in the midgut tissue. Therefore the results, given in Fig.1, only concern activity of the gut contents.

In the gut of freshly hatched bees the enzymatic activity is low, but increases in the first 16-24 hours to the 3.8 fold level. Activity reaches a maximum at an age of 8 days when the bees are nursing and decreases in foragers.

Bees hatched in September started foraging later - about at the 25th day - than those hatched earlier in the summer. The 20 day old bees have proteolytic enzyme levels which are significantly ($p < 0.01$) higher than those of bees in July. The hypopharyngeal glands were also larger in 20 day old bees in September than in July.

The amounts of protein of the midgut content (only the endoperitrophic space is considered) vary according to age and food consumed: they increase from $26.2 \pm 1.8 \mu\text{g}$ protein/bee in freshly hatched bees to $152.4 \pm 6.8 \mu\text{g}$ in nursing bees and decrease again in forager bees to $73.4 \pm 5.3 \mu\text{g}$. The concentration of soluble protein reaches approximately 5% of fresh weight in the endoperitrophic space in nursing bees and is less in the bees of other ages.

Both parameters, the caseinolytic activity and the amounts of soluble protein in the gut contents, show similar changes with age in the different series and were positively correlated ($p < 0.001$).

The caseinolytic activity seems to be well coordinated to the protein intake und protein need.

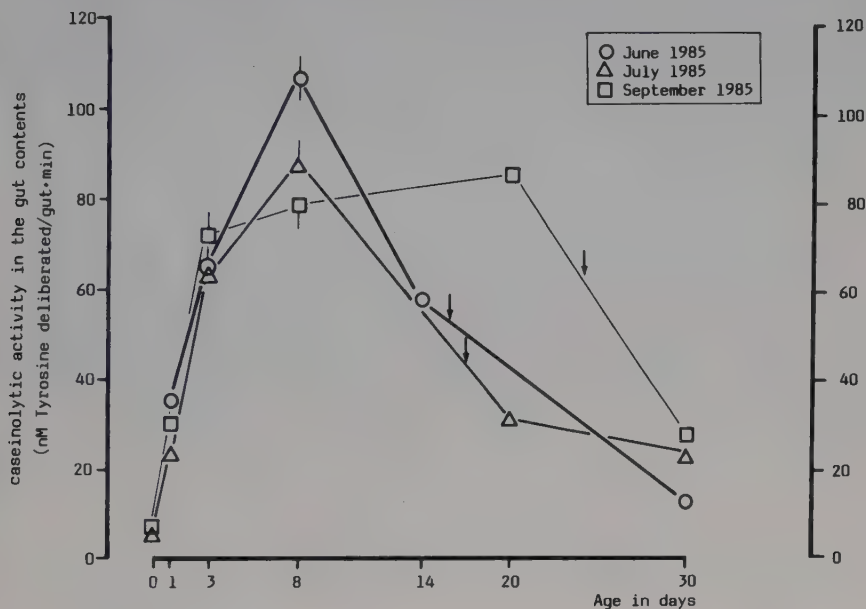


Fig.1.-- Caseinolytic activity in the midgut contents of worker honey bees of different ages from early to late summer were investigated. The means and the SEM are shown. Each point represents 4-6 samples, containing 5 midguts each. The begin of foraging activity of the bees is indicated by arrows.

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Caste Differentiation in Stingless Bees: a Comparative Analysis of Larval Food from 7 Species

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The development of efficient techniques for the artificial rearing of queens in a number of species of stingless bees has proven that caste differentiation in the tribus Trigonini is dependent on the quantity of larval food consumed during larval development. In some cases circumstantial evidence, such as difficulties in artificially rearing queens or the production of miniature queens from worker cells under natural conditions, has opened discussion on qualitative aspects as well. In spite of its essential role in caste development, however, neither the composition of larval food of stingless bees - except for the aberrant species Trigona hypogea - nor the feeding program of larvae have received much attention so far.

Material and Methods

In the present study water, sugar, protein and free amino acid contents, as well as the free amino acid composition and the polypeptide patterns of worker larval food were analysed for the following species: Scaptotrigona depilis, Scaptotrigona bipunctata, Nannotrigona testaceicornis, Partamona cupira, Tetragonisca angustula, Plebeia droryana and Melipona quadrifasciata. Larval food samples were collected from brood cells soon after oviposition. For larval food of the species S. depilis the seasonal variability was assessed, and the larval feeding program was analysed.

Results

I. Feeding program of Scaptotrigona depilis worker larvae:

- a) Total composition: The water content varies between 40 and 50 %. After oviposition until L4 the sugar content drops from 13 to 7 %, and increases again in L5 to 10 %. The protein and free amino acid contents drop more or less continuously during larval development.
- b) Protein composition: With the exception of three bands in the higher molecular weight range (70-85 kd) the protein pattern remains stable during larval development. The polypeptide patterns of queen and worker larval food did not reveal any caste specific differences.
- c) Free amino acids: The absolute concentrations of most amino acids diminish with increasing larval age. With respect to their relative proportions no general trend was observed. The seasonal variability of the free amino acid composition is considerably high.

II. Comparative analysis of meliponine larval food:

- a) Total composition: The water content of meliponine larval food with values between 40-50 % is distinctly lower than that of honey bee larval food, whereas the sugar content for all species lies within the same range as that for Apis mellifera, i.e. between 5-12 %. Each genus has a characteristic protein pattern. Five protein bands (on SDS-polyacrylamide gels) are present in the larval food of all species. Each species has a characteristic free amino acid composition. In all species proline is the dominant free amino acid.

Changes in the Content of Some Substances in *Formica aquilonia* Yarr. in Summer

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Changes in the fat, glycogen, glucose and free amino acids content in *Formica aquilonia* from the beginning of June up to the end of September were studied. Workers from two different parts of the ant-hill were collected and analysed separately: the first group - ants from the top of the ant-hill, the second group - ants from the deep chambers under the ant-hill. The methods used were described earlier (Hansen, Viik, 1979, Hansen et al., 1982).

RESULTS

The fat content in the ants of the first group was relatively low and ranged between 1.7 and 2.9 per cent of the fresh weight, while that in the ants of the second group was 4.2 - 9.0 per cent. The maximum level was observed in July and the content decreased significantly by the end of August. In June and July the content of carbohydrates was low and sharply increased in August in the ants of the second group. The glucose content rose from 0.4 to 2.2 per cent and the glycogen content from 0.3 to 5.6 per cent by the end of September. Thus, the preparation for hibernation began already in August. But in the ants from the hill top the content of glucose and glycogen remained on a low level (below 0.8 per cent). The ants from the deep chambers under the ant-hill contained more free amino acids than the others, maximum level 7.0 and 3.7 mg/g respectively. The maximum content was observed in July and it decreased twice by the end of September.

It can be concluded that there occur certain differences in the concentration of the studied substances in the individuals collected from different parts of the ant-hill. During the whole summer there are workers with a great amount of reserve substances like "live depôts" in the deep chambers under the ant-hill.

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2.2 Neurobiology, Memory and Learning

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Memory Traces in the Honeybee

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Honeybees are fast and perfect learners at the feeding place. Even after one learning trial on a color or an odor signal they change their search behavior for many hours, and after only 3 learning trials they keep the memory for a life time. Lindauer (1963) observed bees at the same feeding place after more than 3 months of winter rest. This long lasting memory is the result of very short learning trials. The exposure to a color or an odor signal for 3 sec just before a short (2 sec) reward with sucrose is sufficient to produce a stable and long lasting memory. It is, therefore, likely that the learning trial triggers an internal neural storage mechanism, which processes the association between the signal (conditioned stimulus, CS) and the reward (unconditioned stimulus, US) in such a way that a long lasting memory trace results. We have attempted to characterize this hypothetical "central automatic memory processor (cAMP)" by determining the temporal dynamics the different phases of the memory trace, their behavioral effectivenesses, their genetic basis, and their contents.

METHODS

Honeybees (*Apis mellifera carnica*) were trained either as free flying animals to color marks at a feeding station or conditioned to odor signals as fixed animals (see Menzel, 1985). Color trained animals were tested in a dual forced choice test situation (chance behavior 50%). The fixed bees were first tested to the odors prior to training, and the spontaneously responding animals (less than 5% in most cases) were not included in the test group (chance behavior 0%).

RESULTS

The dynamics of the memory trace.

If bees are trained 3 times or more they respond to the learned stimulus to a very high and constant probability independent of the time of the test. If the animals are trained only once they show a bi-phasic time dependence of the probability of correct responses: the level of correct responses is high immediately after the learning trial, deteriorates within the first few minutes and climbs up to the same high level as initially at longer intervals (≥ 4 min). Reversal learning experiments reveal that the first period shortly after learning is divided in two distinct

phases, the first minute during which the memory is very effectively erased by an additional controversial learning trial, and the following 2 minutes during which reversal learning is not effective. More behavioral experiments using the resistance to extinction as a measure for the stability of the memory trace give the same division of an early memory phase into two separate periods.

We know from earlier experiments (Menzel et al., 1974, Erber, 1975, Erber et al., 1980) that the memory following one learning trial is highly sensitive to experimental procedures like cooling, narcosis or weak electrical stimulation (EBS) of the brain, if these procedures interfere with the memory within the first 3-4 minutes. Most interestingly, very little retrograde amnesic effect is found, if several massed learning trials are given in the first 30 sec and EBS is applied immediately. A more detailed analysis using the olfactory conditioning paradigm shows that additional CS's or US's alone do not prevent retrograde amnesia, but only additional associative events. Furthermore, it can be shown that an additional learning trial facilitates the transfer from a susceptible form of memory into an unsusceptible memory. If an amnesic treatment follows two quick learning trials then the content of the second learning trial is lost, but that of the first is saved.

Selection of good and bad learners: differences in the dynamics of the memory phases.

Brandes (in press) has recently selected good and bad learners, and bred them first parthenogenetically, later sexually by internal crosses. We tested the two lines in a free running recall experiment after one trial conditioning to an odor stimulus. The good learners have the well known bi-phasic time course with a somewhat speeded-up early memory phase. The bad learners don't respond initially less to the CS than the good learners. Later the memory deteriorates quickly. We conclude that the bad learners are effected in the process which is responsible for the transfer from an early, labile memory form into a late stable memory form.

The content of the early and the late memory trace.

We may expect that the consolidation of the early memory trace into the late memory trace may also change the content of the memory and not only its control over behavior as pointed out above. But how can we measure the content of the memory? One way is to ask, whether the generalization gradient with respect to several odors is different immediately and later after one trial odor conditioning trial. Smith (unpubl. results) conditioned the bees to pentanal or to citral and tested after 30 sec or after 15 min the responses to pentanal, citral, geraniol, 2-pentanol. Each bee was tested only with one of the four odors and only at the short or the long interval. The generalization gradient turns out to be significantly steeper at the longer intervals after training of pentanal, whereas no differences were found after training to citral. Pentanal is a floral odor, citral a prominent component of the pheromone of the Nasanov-gland. It is tentative to speculate that the

pheromone is a specified or labeled stimulus, whose neural representation is highly prepared for appetitive associations. The memory trace may thus reach a final form immediately. In the case of the floral odor one might argue that additional processing enhances the profile of its neural representation and thus favours sensory discrimination with the late memory trace.

Attempts to localize the memory traces.

Any search for the neural correlates of the memory traces or the transfer mechanisms between them needs to know where in the brain one should search. We know from local cooling experiments (Erber et al., 1980) that the transfer process from the early to the late and stable memory can not reside in the sensory neuropil of the antennal lobes, nor does it need a functional circular circuit between the antennal lobes, the mushroom bodies and the lateral protocerebrum. The mushroom bodies, however, are essential structures for the establishment of a permanent memory trace, because their cooling eliminates the memory trace with the same time course as cooling of the whole animal. Sugawa (unpubl. results) repeated these experiments using differential ac stimuli across one alpha lobe as the amnestic treatment. The animals were conditioned by one trial to an odor stimulus, which was presented only to one antenna. She found that an ac stimulus at the ipsilateral alpha-lobe erases the memory trace with the same time course as cooling or electric stimulating of the whole animal. Stimulation of the contralateral alpha-lobe, however, reveals a bi-phasic time course with no amnestic effect during conditioning and shortly (3 sec) afterwards, but a significant amnestic effect 3min after conditioning. We conclude that the contralateral alpha-lobe of the mushroom bodies participates at the establishment of a stable memory trace at a later time, but not shortly after conditioning. This result is particularly interesting, because bees never respond to the conditioned stimulus if it is presented to that antenna, which was not stimulated during conditioning.

CONCLUSION

Our results are consistent with a model that assumes a time and event dependent sequential structure of memory stages. These memory stages are characterized by their time course in free running recall tests, their differential control of choice behavior, their sensitivities towards associative events and retrograde amnestic treatments, their distribution in the brain and their separation by genetic selection. Furthermore, the memory content may be different in the early and late memory stages.

The sequence of memory stages is an adaptation to the sequential choice behavior during foraging bouts. Approaches to flowers within a floral patch follow each other with an average interval of several seconds, and choice behavior is thus controlled predominantly by the very first memory stage, the working memory. Foraging bouts are separated by a few

to many minutes. Thus, when the bee arrives back at the same floral patch the earlier learning will be consolidated in a permanent memory, even if she had only visited a single flower with unlimited nectar.

A multiple memory trace system may not be a unique feature of the bee brain. However, the actual temporal dynamics and the particular qualitative and quantitative properties are obvious adaptations of a flower visiting species to the general pattern of food encounters under natural conditions.

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SYMPOSIUM

Neurobiology, Behaviour and Learning in the Honey Bee

Organizer: Randolph Menzel

Introduction

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The honeybee has become a model system for neuroethological research par excellence. No other insect species is so well known with respect to individual and social behavior, sensory capacity, orientation, communication, stereotyped and plastic behavior. When von Frisch carried out his famous training experiments with bees more than 70 years ago, the honeybee had already attracted many serious scholars of animal behavior and even more naturalists and curious apiculturists e.g. Sprengel (1793), Darwin (1876). Forel (1910) and Buttel-Reepen (1900) already knew many details about the social organization of the bee colony, the co-evolutionary relationship between flowers and the bee as a flower visiting insect, and about the orientation and learning in bees. Von Frisch (1914, 1919, 1965) incorporated this knowledge into his most ingenious experiments, and thus the honeybee was established as being a wonderfully rich source of information with regards to animal behavior and sensory capacity. Von Frisch once compared the honeybee to a magic well, in that "The more you draw from it, the more there is to draw!", and even today this well has still not dried up. The emphasis and methods in specific areas of research have of course changed since those early experiments, but the basic questions remain the same, namely 1) What is the animal able to perceive and do? 2) Which sensory and central nervous mechanisms control these actions? and 3) How does the animal adjust its behavior to the changing needs of the whole colony?

New and exciting insights into the bee's perceptual capacities have been acquired by combining von Frisch's training technique with sophisticated experimental arrangements. Werner reports in her contribution that bees have color constancy that is not inferior to that observed in humans. She uses an experimental arrangement (Land's "Mondrian") which permits the quantification and manipulation of all the visual parameters necessary to perform such a conclusive experiment. Wehner and his co-worker Rossel have recently settled a long debate about the ability of bees to orientate towards the pattern of polarised light. Additional information is provided by Edrich's contribution in this symposium. Once again, the break-through resulted from using a combination of complicated experimental set-ups with more traditional behavioral tests. A third example is Kevan's recent demonstration (Kevan and Lane, 1986) that bees perceive sub-microscopic surface structures which help to guide the bees to the nectar source in the flower. The dance performance of bees has recently been studied on a comparative level, and such investigations have shown the importance of both gravitational and visual cues (see Dyer's contribution). Seeley in his plenary lecture has added a new dimension to the study of information transfer within the bee colony, namely the capacity of a colony to become a "high-level cognitive entity" by gathering, processing and distributing information. The complexity of the neuronal machinery subserving such a capacity has, unfortunately, forced a temporary capitulation among neurobiologists, but hopefully a better understanding of the components of this behavior and their respective neural substrate will eventually lead to a comprehension of social information exchange. Present experimental work is geared towards answering questions such as 1) How do bees measure distance? 2)

How is the individual effort for food collecting measured? 3) How is the quality of the food determined? and 4) How are the requirements of the colony recognised by the individual bee? (see Seeley, Waddington, Schmid-Hempel in other symposia).

The most active neurobiological research is directed towards a functional analysis of the neuroanatomical structures of the bee brain. The distribution of several neurotransmitters and neuromodulators have been localised by immunocytochemical techniques (see the papers by Schäfer and Bicker, Schürmann and Elekes, Noble and Goodman, Erber, Mercer). The quality of this research sets the pace for neuroanatomical studies in insects and it is in this area of research where new insights into the function of the brain may be expected.

The thin central neurons have for a longtime prevented a careful neurophysiological analysis of the neuronal mechanisms that underly certain behaviors in bees. However, advanced recording techniques have been developed with the intention of overcoming these restrictions, and the articles by Goodman, Erber and Hombers, Mertel and Maronde, and Pomfrett illustrate the exciting nature of the evidence collected from such studies. The intracellular recordings are always combined with intracellular stainings, and these have resulted in particularly fascinating photographs of stained individual neurons that are surrounded by the neuronal meshwork of the central nervous system. The accumulation of such important information will undoubtedly help us to understand at least partially the neuronal basis of certain behavioral acts such as fast reflects (e.g. flight control, proboscis extension, head movement) and rhythmic behavior e. g. the diurnal rythm of activity and rest (see Kaiser).

Developmental studies on honeybees have for a long time been neglected, particularly with regards to neuroendocrine regulation and behavioral responsiveness. The contributions by Robinson, Masson, Arnold, Gascuel, Fonta, and Pham-Delegue show that such studies on the honeybee contribute important knowledge to general questions dealing with the successive development of behavior in adult insects.

The ability of the honeybee to adjust its behavior to new environmental conditions by fast and effective learning processes, clearly disproves the notion that insects are stereotyped robots whose behavior is controlled by fixed programmes. On the contrary, the surprising capacity of the honeybee brain becomes more apparent as one begins to unravel the mysteries that surround the behavioral and neuronal basis of learning and memory. I point out in my contribution, that certain general features of a learning system (e.g. the succession of memory phases) are also found in the bee, but that particular aspects of this system have become specifically adapted to the ecological restraints experienced by an animal which collects small amounts of food from a relatively large number of unpredictable food sources. Here again, the bee may prove to be an ideal model that helps to bridge the gap between the traditional attitude of the learning theorists, who argue for general laws in learning, and the ethological approach which emphasises species specific adaptations in learning and memory. The most interesting insight into the genetic components of associative learning have been provided by selection studies on good and bad learners (Brandes). Although a social animal is a particularly difficult subject for genetic studies, it does also offer great advantages e.g. the study of parthogenetic development in females of the Cape bee.

Behavioral and neuroethological research began with the work of von Frisch 70 years ago. It has continued with sophisticated sensory, physiology, receptor physiology, neurophysiology, neuroanatomy and behavioral analysis. The

papers presented at the two symposia "Neurobiology, Memory, and Learning" and "Neuroanatomy of the Bee Brain" highlight selected examples of recent research work.

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The Evolution of Honeybees

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EVOLUTION OF THE MAIN SUBFAMILIES OF APIDAE

Recent honeybees can be traced back to polymorphic bees of the Baltic amber (Eocene) which were named *Electrapis* Cock. although they have nothing to do with the present genus *Apis*. They combine meliponoid, bomboid and apinoid characters in various ways (Winston and Michener 1977). Three subgenera *Electrapis* s.str., *Protobombus* and *Roussiana* according to the similarity to *Apinae*, *Bombinae* and *Meliponinae*, resp., were established (Zeuner and Manning 1979). Some species of *Trigona*, but no true *Apinae* or *Bombinae*, were found in the Eocene. Therefore, the *Electrapis* group of the early Tertiary can be regarded as the common gene pool for all present Apidae.

A specific epoch of major taxonomic radiation can be established for each subfamily: early Tertiary in *Meliponinae* (Wille 1980); late Tertiary in *Bombini* (Williams 1985); *Pliocene-Pleistocene* in *Apini*, as will be demonstrated later. This time difference of evolution is reflected by rank differences of the taxa: *Meliponinae* - 18 genera, more than 300 species; *Bombini* - 3 genera, about 290 species; *Apini* - one genus, four species (partly with many subspecies).

EVOLUTION WITHIN APINAE

The true *Apis*-type is first found in the lower Miocene (Rhenian brown coal of Rott near Bonn, 25 MY). It was named *Synapis henshawi* by Cockerell 1907, later classified as a subgenus of *Apis* with the three species *henshawi*, *dormiens* and *kaschkei* (Zeuner and Manning 1979). From the upper Miocene (*Sarmatian*, 12 MY) are the fossil bees of the *Rand-ecker Maar* and of *Böttingen* (SW Germany), both classified as *Apis armbrusteri*.

A clear trend in changes of wing venation is observed from *Electrapis* to *Apis mellifera*. In order to estimate the changes in wing pattern numerically, methods of morphometric honeybee taxonomy were applied by joint factor analysis of fossil and recent Apidae using data of 16 wing venation angles. Results: Three compound clusters are visible in the graphic representation (fig.1): a)-*Synapis*, *Apis armbrusteri* + *A.dorsata*, b)- *A.florea* and c)- *A.cerana* + *A.mellifera*. The common cluster *Synapis*+*A.dorsata* shows that wing venation did not change much since the early Miocene, (25 MY). Since wing venation reacts rapidly to ecological and behavioral changes it can be assumed that the Miocenic *Apis* species were open-air nesting in subtropical Europe as is the present *A.dorsata* in S Asia. The same analysis (fig.1) shows an eruptive evolution of the two temperate zone species, *A.cerana* and *A.mellifera*, evidently in close relation to an enormous expansion of their area. The statistical distance of these species to *A.armbrusteri* is distinctly larger than the difference between the two Miocenic species *Synapis* and *A.armbrusteri*. A further increasing speed of diversification is observed at the subspecies level of *A.mellifera* and *A.cerana*,

that is within the last 50,000 years.

The joint morphometric analysis of fossil and recent *Apis* species indicates two major steps in evolution: the first in the early Tertiary, when the *Apis*-type of wing venation appeared, very likely with biological characteristics of present open-air nesting species (e.g. vertical wax combs with hexagonal cells, swarm cluster formation, dance communication). The second step (multiple-comb cavity nesting, temperature homeostasis, wintering ability) before or at the beginning of the Pleistocene represented by the *mellifera*-type of wing venation.

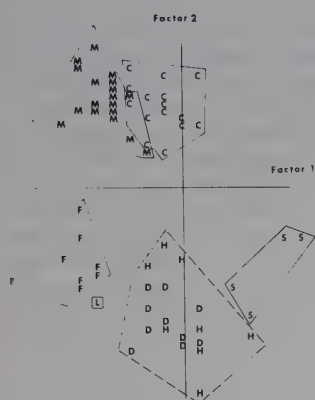


Fig.1. Factor analysis of *Apis* species. C *cerana*; D *dorsata*; F *florea*; H *armbrusteri*; M *mellifera*; S *Synapis*.



Fig.2. Postglacial expansion of Mediterranean *Apis* species. Hatched: maximal glaciation.

EVOLUTION AND RADIATION WITHIN *APIS CERANA* AND *APIS MELLIFERA*

A.mellifera and *A.cerana* are two closely related allopatric species without premating barrier. Radiation within the species occurred during the Pleistocene as can be clearly demonstrated within the Mediterranean area. At least 13 geographic races (subspecies) had been isolated in favored biotopes during the last glaciation. Some of them spread far to the north in the postglacial period: *A.m.mellifera* to NW and NE Europe, *A.m.carnica* to the eastern Alps and the Carpathians, *A.m.macedonica* as far east as the Ukraine (fig.2). The evolution of physiological and behavioral traits as thermo-homeostasis, cavity nesting and perfection of the communication system resulted in an extraordinary expansion and recent radiation of two honeybee species.

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New Perspectives on the Dance Orientation of the Asian Honey Bees

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Early studies revealed striking differences in the way the three honey bee species of Asia orient their waggle dances (Lindauer, 1956). Although *A. cerana* resembled the European bee *A. mellifera* (von Frisch, 1967 review) in being able to dance either on a horizontal (matching their orientation to celestial cues with that remembered from the flight to food) or on a vertical comb in the darkness of the hive (transposing light and gravity according to the "up = towards the sun" rule), *A. dorsata* and *A. florea* dancers seemed more restricted. *A. florea* seemed to orient only to celestial cues, and only while on horizontal portions of its exposed comb. *A. dorsata* apparently used gravity on a vertical according to the transposition rule, but had difficulty dancing when shaded from the sky. In studies in India, I have uncovered hidden complexities in the dances of these species when celestial cues were hidden to the bees on the flight or during the dance, or were otherwise altered.

RESULTS

It now appears that *A. dorsata* closely resembles *A. mellifera* and *A. cerana* in being able to dance without a view of sun-linked cues during either the flight or the dance. I observed foragers to fly from a nest in large numbers on moonlight nights, and to dance on their return. Although the light of the moon was apparently necessary for flight, the dances were not referenced to the lunar azimuth. Instead, the bees danced as if to the hidden sun, perhaps on the basis of its position before sunset relative to features of the landscape also visible by moonlight, using an extension of a time-referenced solar memory like *A. mellifera* and *A. cerana* use on cloudy days (Dyer, 1985a).

A. florea can also dance without a view of celestial cues (Koeniger et al., 1982), contrary to Lindauer's (1956) preliminary finding. I have now determined that dancers denied a view of the sky from the top of the nest orient to prominent landmarks nearby (Dyer, 1985b). Bees oriented faithfully to an artificial landmark panorama even when I shifted it relative to geomagnetic and topographical references. *A. florea* also orients well on overcast days, indicating that bees can probably locate the sun's azimuth en route to the food by reference to the landscape. For the dances to be meaningful, dancers and dance followers both must also be able to locate the sun's position relative to the completely different panorama of landmarks visible from the nest.

Further complexities are revealed when A. florea dancers are forced to dance on the vertical sides of the colony with a view of the sky. I rotated a colony so as to expose the dancers on one side to different views of the same patch of sky. The bees' orientation depended only upon the position of the vertical plane in relation to the direction of flight. On the slope facing the direction of the food bees oriented waggle runs downward; on the slope facing away they oriented upward; and on planes parallel to the direction of flight they inclined the waggle runs relative to the direction of the slope as if trying to "point" toward the food (Dyer, 1985b). The same pattern of orientation can be seen in dances on most natural colonies, as dancers move over slopes of various inclinations on the vaulted nest. To take one example of the contrast between this pattern of orientation and that exhibited on slopes by other honey bee species during orientation to gravity or to celestial cues, if visible (as inferred from studies on A. mellifera; von Frisch, 1967), imagine bees flying in the direction of the sun to a food source and returning to dance on a vertical slopes facing the same direction. According to either the transposition rule or a simple matching mechanism the dancers should orient upward, but A. florea would dance downward, hence with a completely different view of the sun. A. florea seems to reference its wagging angle always to the horizontal world even when on a slope. If one projects to the horizontal plane the vector described by any waggle run of a dancing bee moving over various non-vertical slopes, its orientation is the same as that of a waggle run actually performed on a horizontal surface. Thus, a bee dancing on different inclines must somehow compensate for the different views her eyes have of celestial references.

These results show that A. dorsata, A. mellifera, and A. cerana share a very similar system of dance orientation, and that A. florea's system is not simply a restricted, primitive version of that seen in the three other species. Instead, it has diverged from the others, resulting in fundamental differences in the way celestial and gravitational information is processed. These findings thus pose a new set of mechanistic questions and clarify the interspecific differences that any evolutionary speculations must address.

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Effects of Bidirectional Selection on Learning Behaviour in Honeybees (*Apis mellifera capensis*)

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Worker bees of *Apis mellifera capensis* differ from other honeybee races because they lay a high percentage of diploid eggs in queenless colonies (thelytokous parthenogenesis). Depending on the type of food *Apis mellifera* eggs will develop into either workers or queens. Because of this fact, cape worker bees can be used directly in selection experiments.

Bidirectional selection for learning was done as follows:

Conditioned behavior of worker bees was measured and single worker bees with high or low learning scores were identified. Eggs from these selected workers were used to rear queens. Some of the queens, when unfertilized, produced drones which then were used to inseminate other queens also reared from selected workers. Finally, worker bee progeny from the fertilized queens were used to quantify response to selection and to select another generation. After three generations of selection, the resulting high and low learning lines were tested in two other learning paradigms.

In the selection experiments, learning was tested with classical conditioning of the proboscis extension reflex (PER). The test was composed of three blocks. In blocks A and B excitatory conditioning of the PER was produced using a discriminative procedure. Thus, one conditioned stimulus (CS+) was paired with the unconditioned stimulus (US), sucrose, while the second conditioned stimulus (CS-) was explicitly unpaired with the US. Block C was designed to extinguish conditioned responses to the CS+ in block B; bees received the CS+ of block B without sucrose stimulation. Sucrose (US) was presented to a bee by touching its antennae with a small drop of 1.5 M solution. Usually, a PER ensued, and then the bee was allowed to imbibe the sucrose. Two odors were used as CSs -- violet and geraniol. Each CS was delivered in a stream of air that was established after placing a bee in front of an exhaustor system. Since the bees can associate reinforcement with the cues generated by placing them in front of the exhaustor system, "place"-ment alone (without odor) also was used as a CS. In block A, place alone was the CS+ and place+violet was the CS-. In block B, place+geraniol was the CS+ and place alone was the CS-. In block C, place+geraniol was the lone, unrewarded CS. the total number of PERs to the CSs in all three blocks was used to select high and low learners.

Significant differences between the high and low lines were produced after one generation of selection. To further characterize these differences, worker bees from the third selected generation were tested in outdoor experiments. Colonies were established from individual, selected queens. Then worker bees from these colonies were trained in two colour conditioning tests, one at a feeding site 300m in front of the hive the other at the hive entrance.

In the first learning test, a group of marked bees was trained to forage at the artificial food site. Eventually, an unmarked recruit bee arrived at the site and was marked uniquely. Afterwards, all other bees arriving at the food site were trapped preventing them from returning to the hive during the course of the experiment. The lone, marked recruit was rewarded with sugar water in white dishes during the first three visits to the food site. On the bees fourth visit, it was presented simultaneously with two empty dishes, one yellow and the other blue. For two minutes, the number of "tipping-reactions" toward each dish was recorded. Then, these two dishes were replaced with a yellow dish containing sugar water. A complete learning test consisted of eight such trials.

To measure learning at the hive entrance, colonies were accustomed to an uncoloured hive entrance for at least four weeks. Then the hives were placed behind a white wall containing the entrance to the hive. After one day the bees were given a choice between two hive entrances-- one marked yellow and the other marked blue. The number of bees entering each opening was recorded for 30 minutes. After this test for spontaneous color preference, the two "test" entrances were covered and bees were permitted to enter the hive through a single yellow "training" entrance. Then, for 30 minutes on each of 10 successive days, the training entrance was covered, the blue and yellow test entrances were uncovered, and the number of bees entering each was recorded.

Learning tests at the hive entrance and the feeding site were done with four selected lines. Conditioning of the PER produced maximal performance levels of 56%, 49%, 39%, and 20% for line 3,1,2 and 6, respectively. The same rank order of performance levels was obtained from the field tests. Food site learning produced maximal performance levels of 82.4%, 82.2%, 73.6%, 68.9%. After correcting performance levels in hive entrance learning for spontaneous color preference, the four selected lines produced scores of 25.7%, 20.1%, 15.3% and 4.2%.

Current experiments are characterizing the effect of selection on memory, general activity and motivation levels. Results to date indicate that flight activity levels at the food site do not correlate with conditioned PER levels among the selected lines. Supported by the Deutsche Forschungsgemeinschaft (Br827/1).

Behavioural Analysis of Olfactory Learning and Memory in *Apis mellifera*

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It has been recognised for many years that the honeybee has some ability to acquire and store information relating to a variety of environmental parameters, and even today workers are continuing to extend the range of known stimulus modalities (e.g. Walker and Bitterman, 1985). It is widely inferred that the learning capacity of *A. mellifera* has an important role to play in the overall social behaviour of the species. However, the variables which affect learning and the processing and integration of acquired information into subsequent behaviour have received little attention, particularly when compared with that afforded to vertebrate counterparts. The proboscis-extension conditioning paradigm (Takeda, 1961) is a suitably accessible technique for studying such phenomena.

METHODS

The underlying classical conditioning technique is similar to that used by previous workers (Mercer and Menzel, 1982; Bitterman, 1983) in that immobilized insects are presented with training stimuli in certain temporal sequences such that the proboscis-extension reflex response may be taken as a conditional measure of behavioural modification. A progressive acquisition curve for the twenty or more bees comprising a given experimental group provides the basis for analysis. Computer controlled synchronization and delivery of stimuli facilitates the precision and repeatability of experiments.

RESULTS

Conditioning was supported only when the CS and US were presented such that the onset of the CS preceded that of the US (delay conditioning). Other sequences proved ineffective, including those which might have given rise to temporal conditioning, sensitization or pseudo-conditioning. Behaviour changes may therefore be attributed to association (Bitterman et al., 1983).

Of the stimuli tested, including olfactory, visual and tactile stimuli, only olfactory stimuli were found to be effective as a CS for conditioning the proboscis-extension reflex, although Menzel (1983) reports that it is possible to classically condition fixed bees to a light stimulus if the training period is suitably protracted.

Qualitative CS variation tended to produce odour - specific acquisition curves with characteristically enhanced or depressed rates and asymptotes. Of the twenty or so odours tested all supported conditioning to some degree. The range of odours included pheromone components, floral odours and a number of biologically "neutral" odours such as methyl benzoate. Conditioning to citral was the most rapid and produced the highest level of acquisition, whilst isopentyl acetate produced the lowest rate and asymptote. In general, bees

conditioned to one odour showed little discrimination between a range of unconditioned test odours in this situation. This contrasts markedly with results for free-flying bees in feeding-station choice experiments.

Solutions of both sucrose and glucose were effective as a US in these experiments. No difference was observed in their effect on acquisition. Acquisition curves were, however, subject to variation according to US concentration, particularly between 0 and 25%wt. of solution. However, concentrations reduced the final level of acquisition for a given CS odour, probably being a function of individual levels of motivation. The rate of extinction was also dependant upon the US concentration. Increasing the number of CS/US pairings from one to eight also affected the rate of extinction, even where the final levels of acquisition were the same (with citral normally by the third presentation). In general, the lower the concentration of the US or the fewer the number of CS/US pairings at a given US concentration, the more rapid the extinction of the response.

It was found that acquisition could be inhibited by first pairing the test odour with a noxious stimulus (eg; 15%wt, sodium chloride solution) prior to training with a 25% sucrose solution US.

There is some evidence that bees can acquire side-specific differential US information.

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Preliminary Results on the Neuronal Bases of Odour Memorization by Buccal Gustatory Reinforcement in the Honeybee *Apis mellifera*

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Foraging behaviour of the worker honeybee shows a quick adaptation to quantitative and qualitative variations of food sources. The insect choices rely on associative conditioning between the floral aroma (olfactory conditional stimulus, CS) and the food intake (gustatory inconditional stimulus, IS and reward). In order to analyse the neuronal basis of this highly effective bisensory integration (Menzel, 1967) we first determined the nervous pathways of CS and IS processing. We present here the observations on the central nervous structures which receive inputs both from antennae (olfaction) and from mouthparts (gustation). We used a cellular staining method with Co++ ions and a double staining with Co++ and Ni++ ions to obtain the central network of the antennal and the buccal systems simultaneously.

The subesophageal ganglion (SOG) is the first level of projection for mouthparts fibers. A migration of CoCl₂ from the glossa revealed that a thin bundle of fibers leaves the SOG and goes straight away into the anterior lateral part of the antennal lobe. This projection level corresponds to the anterior and dorsal group of deutocerebral glomeruli (described by Arnold *et al.*, 1985 and this issue) which receive antennal inputs from the T1 antennal bundle. The projections have convoluted-like paths. They have not many ramifications and exhibit varicosities.

The anatomical data reported here point out a possible interaction between antennal and buccal inputs above the protocerebrum (PTC) (known to be the multisensory integration level), in the deutocerebrum (DTC). Functional studies are in progress to analyse the temporal parameters of gustatory inputs in the central processing of olfactory signals. Extra- and intracellular recordings coupled with dye injection are carried out in the population of 2nd order neurons. Analyses at this first convergence level of olfactory and gustatory inputs might partly explain the initial stage of (i) reflex movements of cephalic appendages ; (ii) olfactory mnesic trace stored in the PTC.

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Aspects of Odor Perception in Honeybees

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The work reported here is motivated by questions relating to the role of olfaction in the discrimination of nestmates and kin in the honeybee *Apis mellifera*. Two sets of experiments are discussed. The first deals with the perception of individual compounds in mixtures made up from various pairs of volatile (citral, geraniol, linalool, and limonene) and nonvolatile (un- and dodecanoic acids) compounds (mixture perception experiments). The second deals with the ability of worker honeybees to discriminate between mixtures made up from two compounds combined in different proportions (mixture sensitivity experiments). All experiments employ differential conditioning of the proboscis extension reflex as an assay of the ability of workers to discriminate between two odors (for details see Getz, Brückner and Smith, in press). The results are too detailed to present here in any form but a brief verbal summary.

The mixture sensitivity experiments indicated that worker bees can be trained to discriminate between such closely related compounds as undecanoic and dodecanoic acids as well as tricosane and pentacosane *n*-alkanes. Furthermore, for each pair, workers could be trained to discriminate between mixtures made up of relative proportions of the two compounds, although discrimination was stronger for the *n*-alkanes than for the fatty acids. Since fatty acids and odd-numbered *n*-alkanes are known to be constituents of cuticular waxes in the honeybee, *A. mellifera*, and the relative proportions of each compound may be genetically determined (Carlson and Bolten, 1984), our results indicate that cuticular waxes could provide olfactory cues for kin discrimination within the hive.

The results of the mixture perception experiments indicate that, although worker bees have some ability to associate different mixtures made up from the same compounds, the success of this task depends on the particular compounds used. For example, they seem to be to most easily separate out limonene from linalool. Note that from our results we could not infer that workers are actually able to identify the components of mixtures. Rather, we could only infer that workers can make associations between similar odors.

Although extension of the proboscis is an out-of-context response for kin discrimination based on odor cues, and care must be taken in interpreting results, it is clear that differential training of this reflex can provide valuable insights relating to the question of kin discrimination in honeybees using chemical cues and to mechanisms of olfaction.

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Preliminary Investigation of the Role of Dopamine in Primary Olfactory Centres of the Bee Brain

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The biogenic amines dopamine, 5-hydroxytryptamine, and octopamine have been detected in the antennal lobes of the bee brain using high performance liquid chromatography and radioenzymatic assay techniques (Mercer *et al.*, 1983). Dopamine-containing neurones are restricted to the inner margins of the peripherally located 'olfactory glomeruli' (Klemm, 1976; Mercer *et al.*, 1983), where sensory receptor inputs converge on local interneurons as well as on first order olfactory interneurons that project to the calyces of the mushroom bodies in the protocerebrum of the bee brain. The function of dopaminergic neurones in primary olfactory centres of the brain of the honeybee is not known. Behavioural techniques have been used in a preliminary investigation of the role of dopamine in the antennal lobes of the honeybee, *Apis mellifera*.

MATERIALS AND METHODS

Bees were conditioned to orange scent using the proboscis conditioning paradigm (Kuwabara, 1957). Prior to conditioning the left antenna was isolated from the scent stimulus using a small plastic cap. Dopamine (0.25µl) made up in distilled water to a concentration of 10^{-6} M was applied either to the antennal lobe ipsilateral to the antenna exposed to the scent stimulus during conditioning (the 'conditioned antenna'), or to the antennal lobe contralateral to the 'conditioned antenna'. In control groups distilled water alone was applied to the antennal lobe. The effects of applying haloperidol (10^{-5} M) to the antennal lobe prior to treatment with dopamine was examined also.

RESULTS

55% of bees conditioned to orange scent with one antenna isolated from the scent stimulus responded to the conditioned stimulus 15 minutes after a single conditioning trial. The level of responses in bees conditioned in this manner remained stable for at least 45 minutes. Bees displaying the conditioned response (proboscis extension) 15 minutes after conditioning were used to examine the effects of dopamine on the level of responses to the conditioned olfactory stimulus (Fig. 1). Treatment of the antennal lobe contralateral to the 'conditioned antenna' had little effect on the percentage of bees displaying the conditioned response (Fig. 1). However, application of dopamine to the antennal lobe ipsilateral to the 'conditioned antenna' caused a marked reduction in percent response to the conditioned stimulus (Fig. 1). Applying distilled water alone to the antennal lobe ipsilateral to the 'conditioned antenna' reduced slightly the percentage of bees responding to the conditioned stimulus, but the reduction in response levels following treatment with distilled water is significantly smaller ($p < 0.001$) than that resulting from the application of dopamine to the

antennal lobe. The difference between the effects of applying dopamine to the antennal lobe ipsilateral or contralateral to the antenna exposed to the scent stimulus during conditioning is highly significant also ($p < 0.001$).

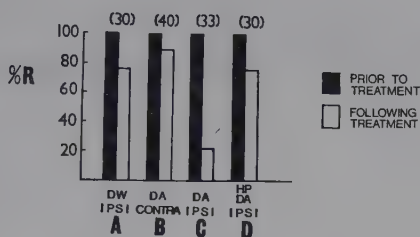


Fig. 1. -- The effects of dopamine ($10^{-6}M$) applied to the antennal lobe of the brain on the percent response (%R) to a conditioned olfactory stimulus. All bees displayed the conditioned response prior to treatment. (A). Distilled water (DW) applied to the antennal lobe ipsilateral (IPSI) to the 'conditioned antenna' in a control group. (B). Dopamine (DA) applied to the antennal lobe contralateral (CONTRA) to the 'conditioned antenna'. (C). Dopamine applied ipsilateral to the 'conditioned antenna'. (D). The effect of applying haloperidol (HP) to the antennal lobe immediately prior to treatment with dopamine. Number of animals tested in each group is given in brackets in the diagram.

The effects of dopamine on percent response to a conditioned olfactory stimulus are reduced significantly ($p < 0.001$) by prior treatment of the antennal lobe with the butyrophenone, haloperidol (Fig. 1).

It is possible that dopamine-containing cells form a population of inhibitory interneurons that influence output of sensory information from the antennal lobes to the protocerebrum of the brain in the bee. Inhibitory responses of deutocerebral neurones to olfactory stimuli have been observed in this insect (Homberg, 1984; Flanagan, personal communication). The effects of biogenic amines, and amine antagonists, on response characteristics of deutocerebral neurones in the brain of the honeybee are currently under investigation.

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A Comparison of the Distribution of GABA-, GAD-, and 5-HT-like Immunoreactivity in the Visual System of the Honeybee

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Immunohistochemical investigations of the distribution of serotonin (5-HT) in the brain of the honeybee revealed a distinct pattern of labelled layers in the three visual ganglia: the lamina, medulla, and lobula (Schürmann and Klemm, 1984). We examined the bee brain for γ -aminobutyric acid (GABA)-like immunoreactivity (Schäfer and Bicker, 1986) and found some overlap in the distribution of GABA-like immunoreactivity and 5-HT-reactive layers in the visual ganglia.

In order to obtain independent evidence for the localization of GABA we also examined the distribution of glutamic acid decarboxylase (GAD), the enzyme which is involved in the synthesis of GABA from glutamate, and found that the GABA and the GAD staining patterns are virtually identical.

METHODS

Immunohistochemical staining was performed on paraffin serial sections according to the peroxidase-antiperoxidase (PAP) method. For the GABA-staining we used an antiserum against GABA-KLH conjugates, prepared and characterized by Dr. T. Kingan, Columbia University, N.Y. The GAD antiserum was raised against GAD from rat brain by Oertel et al. (1981). For the 5-HT labelling we used a commercial anti-5-HT (Immuno Nuclear).

RESULTS

GABA- and GAD-like immunoreactivity

GABA-like immunoreactivity was found in all three visual ganglia. We counted ca. 9000 GABA-reactive somata in each optic lobe; this is almost 5 percent of the total number in this neuropile. In the lamina it is restricted to a narrow stratum close to the outer chiasma. In frontal sections, projections of fibers running along the lamina-chiasma border can be seen which can be traced for almost 1/2 of the entire lamina length. Horizontal sections reveal the course of these fibers running through the outer chiasma to their cell bodies which are located frontally to the inner chiasma, or in rare cases in the outer chiasma. Around the outer chiasma numerous cell bodies are labelled, some of which have neurites projecting towards the lamina. They may be amacrine fibers of the am_{i2} type which is the only neuronal type restricted to the innermost lamina layer whose soma position coincides with that of GABA-reactive cell bodies. GABA-reactivity in the medulla shows the most pronounced stratification of the entire optic neuropile. From the density of the staining one can distinguish nine strata. As in the lamina, most of the staining in the medulla neuropile can be attributed to small local interneurons, presumably amacrine fibers, whose somata lie around the outer chiasma. The outermost medulla layer contains a few relatively thick fiber arranged in a regular mesh covering the outer surface of the ganglion. In horizontal sections, the fibers can be traced from the anterior edge of the medulla

to the frontal edge of the lobula where the cell bodies are located in a cluster of labelled somata. In the lobula GABA-like reactivity is also very dense but less stratified than in the medulla. Stained fibers which approach the lobula from the inner chiasma or from the medulla are very thin. They follow the course of the chiasma and enter the lobula at its outermost layer. There are only a few widefield elements stained which leave the lobula through the anterior bundle.

The GAD antiserum revealed essentially the same staining pattern as described above for the GABA antiserum, the only difference being that the cell bodies of the GAD-reactive cells appear slightly more faint and that their nuclei are not stained. It should be noted that the antiserum was raised against GAD isolated from rat brain. It must therefore recognize epitopes which GAD from the rat and bee brain have in common. GABA- and 5-HT-like immunoreactivity

In contrast to the abundance of GABA-reactive cells, there are only a few (ca. 20) 5-HT-reactive fibers in each optic lobe. They are all widefield tangential neurons and are restricted to narrow bands in the neuropile of each ganglion. In the lamina they have a striking similarity with the GABA-reactive tangential fibers: same soma position, axon projection and arborisation pattern. With a GABA/5-HT double labelling on the same section we proved that the GABA- and the 5-HT-reactive cells form two subsets of fibers with projections and arborisations in close apposition to each other (Schäfer and Bicker, 1986b). In the medulla 5-HT-like immunoreactivity is restricted to a single layer in the medial part of the neuropile. The 5-HT-reactive fibers invade at the anterior margin where they wrap around the fibers of the anterior commissure which connects the medulla with the calyces of the mushroom bodies. In the lobula the arborisations of the 5-HT-reactive fibers are limited to three separate layers. The fibers, of which there may be only two, approach the lobula from the posterior protocerebrum.

CONCLUSION

With the exception of a few GABA- (and GAD-) reactive widefield neurons, GABA seems to be located predominately in amacrine fibers and other local interneurons, which may subserve functions such as lateral inhibition between adjacent columnar elements. Serotonin on the other hand is found exclusively in widefield tangential neurons, which may influence synaptic transmission across large neuropilar areas.

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Immunohistochemical Localisation of a Gastrin /CCK-like Peptide in the Brain of the Honeybee

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Monoamine and amino acid transmitter-containing neuronal systems are well described in the honey bee brain (Mercer *et al.*, 1983, Schafer and Bicker, 1986), but little is known about the nature or distribution of neuropeptides. We have used standard immunofluorescence techniques before and after *in vivo* pretreatment with the tubulin-binding alkaloid colchicine to map the distribution of gastrin/CCK-like immunoreactivity. A gold-based immunohistochemical method was used to identify the intra-cellular location of antigenic sites at the EM level.

RESULTS

Large amounts of immunofluorescence were present within the mushroom bodies and central body. In the mushroom bodies, immunofluorescence was arranged in bands within the alpha and beta lobes which projected into the pedunculi as concentric rings (see Mobbs, 1982). None was present in the calyces, or in the columns of Kenyon cell processes projecting into the pedunculi and lobes. Intense immunofluorescence was present in varicose fibres in the ventro-anterior part, and, to a lesser degree, in the dorso-posterior part of the central body.

Pretreatment with colchicine increased the intensity of immunofluorescence in the mushroom bodies and central body, and unmasked gastrin/CCK-like immunofluorescence in other brain regions (Fig. 1). Eleven immunoreactive cell bodies were present in the region of the pars intercerebralis, and seven in the antero-medial brain rind. Fine plexuses of immunofluorescent fibres were diffusely distributed throughout the undifferentiated neuropil of the protocerebrum, trito-cerebrum and sub-oesophageal ganglion. Weak homogenous immunofluorescence was present in the macroglomeruli of the antennal lobes. Immunofluorescent fibres were also present in the ocellar tracts, and gave rise to extensive arborisations in the sub-retinal ocellar plexuses. In the optic lobes, fine immunofluorescent fibres were present throughout the lobula and in three strata of the medulla.

Immunoreactivity at the EM level was identified in the mushroom bodies and central body where it was localised around small (40–60 nm) clear vesicles in synaptic terminals. It was not observed in terminals containing predominantly large dense-cored vesicles or in the inter-terminal segments of varicose fibres.

DISCUSSION

The distribution of gastrin/CCK-like peptide in the mushroom bodies is similar to that described for dopamine (Mercer *et al.*, 1983) and serotonin (Schurmann and Klemm, 1984).

The absence of peptide from the calyces and the characteristic banding pattern of distribution in the lobes, indicates that the peptide is contained within extrinsic mushroom body neurons which form complex input, output and feedback routes in this neuropil. It is also possible that the peptide occurs

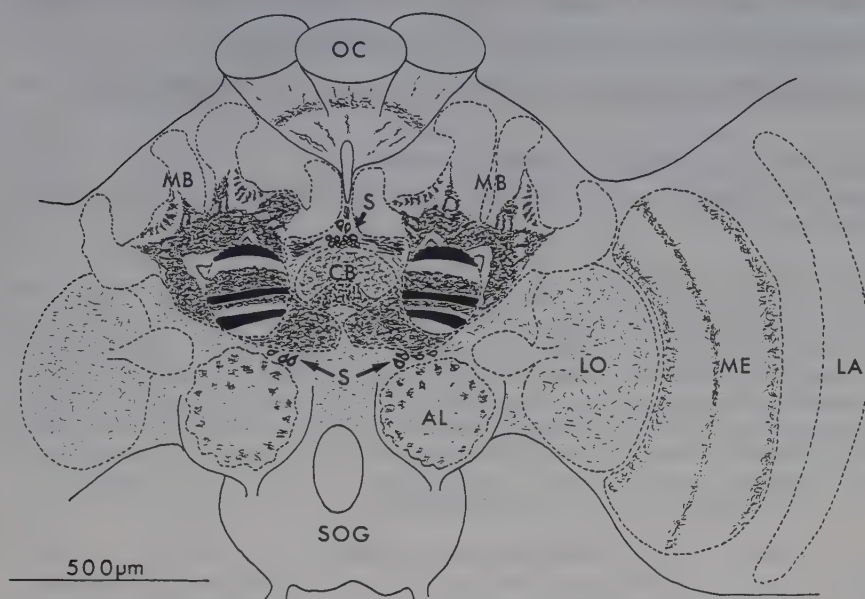


Fig. 1.—Distribution of gastrin/CCK-like immunoreactivity in the honey bee brain seen in frontal view. The density of shading reflects the intensity of immunofluorescence observed following pretreatment with colchicine.

AL, antennal lobe; CB, central body; LA, lamina; LO, lobula; MB, mushroom body; ME, medulla; OC, ocellus; S, cell bodies; SOG, sub-oesophageal ganglion.

within aminergic neurons.

The intracellular locality suggests that the peptide may play a role in neurotransmission, but the exact nature of this role is unclear. Further investigation of the biochemical and physiological properties of this neuropeptide in the bee brain are needed. The application of colchicine has been demonstrated to be an important step in fully mapping the distribution of molecules with nervous systems.

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Synaptic Connectivity in the Mushroom Bodies of the Honeybee Brain: Electron Microscopy and Immunocytochemistry of Neuroactive Compounds

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Though many morphological studies on the mushroom bodies (MB) in the central brain of honeybees have led to a solid ground plan of this prominent area (Mobbs, 1982), the fine composition of their architecture, cell types and synaptic connectivity are not completely described. Modern neuroanatomy can depict details of the MB neuronal network not yet fully accessible by physiological approaches, but useful and important for functional models. By use of conventional electron microscopy and immunocytochemistry of some neuroactive compounds, our study contributes to a more sophisticated view of the bee MB system.

MATERIAL AND METHODS

Adults of worker honeybees, *Apis mellifera* L., were used for staining of single nerve cell types and for conventional electron microscopy (Schürmann, 1974). Immunocytochemistry for serotonin, dopamine and GABA was employed as described elsewhere (Schürmann and Klemm, 1984, Bicker et al., 1985).

RESULTS

The paired MB in the bee brain consist of thousands of Kenyon cells (5 intrinsic cell types) which send their fine T-shaped parallel fibres through the compartments of the calyces to the top of the α - and β -lobes. More than 20 extrinsic cell types connect the MB to various brain locations. Extrinsic and intrinsic fibre arrangements compose distinct subcompartments. The MB receive their main inputs by extrinsic elements converging into the calyces, and the output information is transferred by extrinsic cells mainly in the lobes. Direct or indirect crosstalk between subcompartments and parallel intrinsic channels have been suggested for the columnar stalk and lobes. The peculiar specific geometry of MB fibre elements in bees and a reduced scheme of synaptic connectivity have been considered for functional hypothesis and models (Schürmann, 1974).

As the MB system consists of more than 25 cell types (according to gestalt principles), interconnected in a complex manner, it is obvious that compartmentation and synaptic wiring is not satisfactorily understood from the previous data.

Therefore we have started to study marked MB neurons, chemically defined by their neuroactive compounds (serotonin, dopamine GABA) (Mercer et al., 1983), using immunocytochemistry for coupled light and electron microscopy.

All the three putative transmitters were found in extrinsic fibres which invade known MB subcompartments in a specific manner. Serotonin is distributed throughout the lobes and in some subcompartments of the stalk. Dopamine is found in lip and collar neuropil of the calyces, but missing in the basal ring. It occupies distinct bundles in the

upper stalk and is detected in the lobes. GABA can be mainly attributed to the protocerebral-calycal tract which connects mushroom body parts.

This GABA-ergic tract can be partially considered as a feedback tract (with presynaptic sites in the calyces and postsynaptic elements in the α -lobe), but is supposed to influence subcompartments in the lobes in a differential manner. Presynaptic GABA- and dopamine containing terminals with partial overlap in the calyces give evidence for complex input glomeruli. Serotonin-ergic fibres with pre- and postsynaptic sites at different fibre types are considered as one substrate for local circuits and interaction of intrinsic fibres.

Our immunocytochemical studies on the bee MB complement conventional morphological studies and reveal a more complex supply of the fine subcompartments with different putative transmitters and synaptic connectivity than could be analyzed by previous studies. Our morphological data support the view of the MB as a special synaptic machine with tiny fibre elements densely packed to complex microcircuits. Complex structural design of the small MB subcompartments - assumed to be functionally diverse - demands for future refined experimental investigations.

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Functional and Immunocytochemical Study of the Processing of the Odorant Information in the Antennal Lobe of the Honeybee

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In the glomeruli of the antennal lobe, the olfactory messages undergo an important integration and encoding before being transmitted to the higher centers (Masson and Mustaparta, 1986). Here the neural mechanisms underlying this integration were analysed on the one hand with the method of 2-deoxyglucose (2-DG) autoradiography and, on the other hand with an immunocytochemical study. It was necessary to initiate such studies by analysing the organization of the glomeruli of the antennal lobe (Arnold et al., 1985, 1986).

Autoradiography of 2-deoxyglucose labelled brain sections was used to study the spatial component of olfactory information coding at the antennal lobe level. Autoradiograph patterns were digitally imaged and then analysed with the image analysis systems Ibas II and Tigre.

After a stimulation with a pure odour (in this study isoamyl acetate), the autoradiograph density patterns showed that uptake of 2-DG was not uniform. Uptake was greatest in the ipsilateral antennal lobe. Other regions showed lighter labelling, a few fibers between the two lobes and part of the contralateral antennal lobes. Labelling in the ipsilateral lobe was not spatially homogeneous but was restricted to the peripheral glomerular layer. Furthermore, the glomeruli were not equally labelled. The most labelled glomeruli were situated in the internal part of the antennal lobe, close to the oesophagus.

Immunocytochemical study showed that GABA and its biosynthetic enzyme GAD were abundant in the antennal lobe. GABA immunoreactivity was mainly found in neuronal cell bodies and in their processes in the neuropile, whereas GAD immunoreactivity was found in the cortical layer of the glomeruli where the synaptic connections are localized.

These data, thus support the hypothesis that inhibitory interneurons have a crucial function in the processing of the odorant information at this first sensory level of the central nervous system.

Furthermore, at the higher level of integration, as for example in the calyces of the mushroom bodies, our results showed that some endings of efferent deutoneurons were also GABAergic.

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Neural Signal Processing in the Median Protocerebrum of the Honeybee (*Apis mellifera* L.)

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Large parts of the bee brain are concerned with the processing of sensory information. The various sensory signals are funneled into the median protocerebrum and from there via descending interneurons to motor centers of the thoracic ganglia. The mushroom bodies (m.b.) and the central complex (c.c.) are largely superimposed on the sensory to motor-center pathways in the lateral protocerebrum. Electrophysiological recordings and stainings of single cells, evoked potential measurements, immunocytochemical and autoradiographic investigations have specified our knowledge of neural signal processing in the brain of the bee.

THE MUSHROOM BODIES

The most massive inputs to the m.b. arise from the antenno-glomerular tract (AGT). Most neurons of the AGT display multimodal characteristics, responding to olfactory, mechanical and chemical stimuli applied to the ipsilateral antenna, but these cells can also respond to stimulation of sense organs which are not located on the antenna (Homberg, 1984). In the honeybee, several tracts connect the optic lobes with the calyces. Neurons of these tracts respond to visual stimulation. Some of the cells are movement sensitive and direction selective (Gronenberg, 1984).

In bees, output neurons with arborizations in the lobes of the m.b. often have more complex, multimodal characteristics than calycal input neurons. The pronounced aftereffects of many of these cells are a feature which seems to be characteristic for m.b. output cells.

Feedback neurons of the protocerebral calycal tract (PCT) are characterized by GABA-like immunoreactivity (Bicker et al., 1985). Recent experiments (Hartmann, pers. comm.) with GABA antagonists suggest that the feedback loop is in fact inhibitory and that the aftereffects are not generated by this loop. The application of GABA antagonists also suggests that the calyces are not the only site for inhibitory interactions of this feedback loop.

THE CENTRAL COMPLEX

Several cell types of the c.c. have been studied electrophysiologically (Homberg, 1985). Amacrine cells of the protocerebral bridge respond predominantly to stationary and moving visual stimuli. Recordings from segmental and tangential neurons, however, revealed no systematic differences between these groups in the response patterns to sensory stimuli. Compared to the m.b. neurons, these cells show a lesser degree of multimodality and a relatively high percentage of neurons does not respond to any stimulus. Apparently, the c.c. gets only weak inputs from sensory organs, suggesting that it has some modulatory function within the brain.

THE "DIFFUSE" PROTOCEREBRUM

The lateral protocerebrum is the major area of multimodal convergence of visual, olfactory and mechanosensory inputs which are ultimately transferred onto descending pathways. Most neurons in this area seem to have a unique gestalt and likewise unique physiological characteristics. Therefore, a classification of these neurons may be largely impossible. In the anterior lobes around the m.b., the response characteristics of local interneurons resemble the properties of m.b. neurons and poststimulus aftereffects can be observed frequently.

TRANSMITTER BINDING SITES

With autoradiographic methods we localized transmitter binding sites in the brain of the bee (Scheidler et al., 1986). The binding of (^3H) serotonin and (^3H) octopamine was highly specific (90 - 95%). The highest density of binding sites for both transmitters was found in the m.b. (approximately 160 fmol/mg wet weight).

The distribution of binding sites reveals specific patterns for the two transmitters. The correlation between serotonin-like immunoreactivity and (^3H) serotonin binding sites is excellent in the m.b., whereas there is an obvious mismatch in the c.c.. The distribution of octopamine binding sites is different from those for serotonin in the medulla, the c.c. and part of the m.b..

CONCLUSIONS

We conclude from our studies that temporal signal processing seems to be the eminent function of the m.b.. The different forms of temporal signal processing might be necessary prerequisites for memory formation. The c.c. receives mostly indirect inputs from many sense organs and seems to be involved in the control of information flow between the brain hemispheres.

ACKNOWLEDGEMENTS

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Processing of Visual Information in the Honeybee Brain

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An histological analysis provides the basis for the identification of structures that are presumably important for processing of visual information and are accessible to electrophysiology. This combination of techniques results in an accumulation of information on specific brain neuro-piles, thus allowing us to ascribe specific functions to specific parts of the brain.

MATERIALS AND METHODS

Electrophysiology. Worker bees (*Apis mellifera*) were immobilized in a metal tube. Lucifer yellow filled electrodes (3% aqueous solution) were inserted anteriorly into the exposed brain. A conventional setup for signal amplification, monitoring and storage was used. Optical stimulation was provided by light from a xenon arc lamp projected via ND and interference filters onto the bee, which was centered in a perimeter apparatus.

Histology. Brains were processed according to the method of Steward (1978). After whole mount examination of intracellularly labelled cells, the brains were embedded in plastic and frontal or horizontal sections (25 μ m) were cut. The profiles of visual tracts were analyzed after cobalt injection into selected areas of the brain. The staining was intensified according to the method of Tyrer and Bell (1974).

RESULTS

Of the three optic ganglia, the role of the lamina remains uncertain because of the difficulty of recording from this neuropile. Findings in diptera (Laughlin, 1984) suggest that these neurons may improve the signal to noise ratio of visual input.

Our observations on medulla neurons reveal three main properties: color coding, space contrast analysis, and simple movement detection. The color coding cells show a wavelength-specific reaction; phasic responding cells e.g. may respond with excitation to the turning on of a green light, and the same cell may respond to UV light with an additional excitation when the light is turned off. This reaction is independent of the light intensity. More striking, is a color opponency of neurons that change the frequency of their spontaneous action potential discharge according to the wavelength of the light: long wavelength light leads to a tonic excitation, while UV light inhibits

the cell. Blue light is not coded at all. These neurons have tangentially extending wide dendritic fields that are restricted to a single anaxial stratum of the medulla close to its serpentine layer. Their axons presumably project into the anterior commissure, a large congregation of axons derived from different visual neuropiles.

Neurons involved in space contrast analysis are found in the posterior optic commissure, which consists of about 200 neurites that connect both medullae, crossing the brain at its posterior surface. The single elements are mono- or binocularly sensitive; they show an opponent response in which the position of the stimulus in the receptive field determines the reaction, either excitation or inhibition.

The four neurons of the serpentine optic commissure with their anaxial arborizations cover the whole serpentine layer of both medullae. Additional wide branches extend into each lobula. These neurons are the most sensitive to moving objects of all the cells we have recorded so far in the bee brain. They respond with a strong excitation to movement irrespective of its direction or location in their wide monocular receptive field. Repetition of the same stimulus does not change the responsiveness of these fibers.

A prominent commissure, gathering the axons of cells with dendritic arborizations in the distal lobula neuropile, is the inferior optic commissure (IOC). IOC neurons (about 210 axons have been counted) connect both lobulae, running straight through the brain just above the oesophagus. The IOC is accompanied by lobula neurons which either project to the contralateral protocerebrum or terminate ipsilaterally; some do both. All these cells are very sensitive to the direction of a moving stimulus. In most of these cells this selectivity is expressed by antagonistic responses. One direction of movement leads to tonic excitation, and movement in the opposite direction causes inhibition of spontaneous discharge. This opponency can be restricted to one eye but can also cover the entire visual field. Furthermore, excitation in response to enlargement of the visual angle of a target in the frontal field (equivalent to the approach of an object), and inhibition to its diminution are observed. Some of the neurons with wide dendritic fields in one of the lobulae, which terminate in the lateral protocerebrum, respond to contralateral visual input. This indicates centrifugal connections onto extrinsic elements, presumably via the IOC.

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Motion Sensitive Descending Interneurons in the Honeybee *Apis mellifera*

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Surprisingly, in view of the widespread interest in its visual orientation behaviour, the neural substrate underlying optomotor responses has received little attention in the bee. Directionally selective, motion sensitive units have been identified in the optic lobe (Erber, 1981, DeVoe et al 1982) but the way in which information relative to course correction manoeuvres is signalled to the appropriate motor centres has not been examined. Fletcher *et al* (1984) reported the presence of 12 wide field motion sensitive units in the bee cervical connectives. The response characteristics of these units suggests that they be considered for roles in the control of optomotor behaviour. The ocellar L_D neurons may also be involved in flight stabilisation mechanisms.

METHODS

Intracellular recordings are made from the cervical connectives (CC) while stimulating worker bees either with a moving square wave grating in the frontal visual field or with 2 gratings moving independently on either side of the head. The gratings are moved at 20° or 7° intervals throughout 360° for a period of 3s with a 3s stimulus interval. Cells are subsequently stained with cobalt chloride or Lucifer yellow.

RESULTS

Twelve descending interneurons (DNs) showing tonic or phasotonic responses to wide field-movement have been identified. The cells do not readily habituate and 11 of them do not show adaptation over at least 10s of continuous stimulation. Ten of them show a broad band directional sensitivity to grating movement in the frontal visual field. Their overall preferred direction of movement remains remarkably constant from preparation to preparation and within the same preparation over a period of time although levels of background discharge and bandwidth at the 50% response level may vary. All of the cells arborise in the deutocerebrum immediately posterior to the deutocerebral lobes or in areas at the base of the lobula, and descend at least as far as the prothoracic ganglion. Some have been traced as far as the mesometathoracic ganglion. Many of them arborise in the dorsal suboesophageal ganglion in areas visited by arborisations from tactile hairs on the vertex and by arborisations of neck motoneurons (see CJD Pomfrett, this volume).

The cells have been classified into six groups, DNI-VI, on the basis of their morphology. Anatomically similar cells within the same group do not necessarily share the same directional sensitivity, for example, Group DNII contains 2 cells, one sensitive to vertically

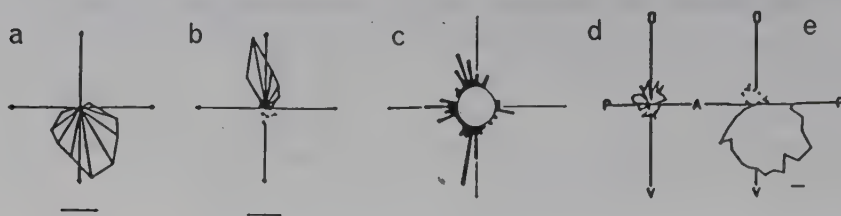


Fig.1. Response of DN II₁ (a) and DN II₂ (b) to motion in 18 directions. Bars 10 Hz. Up = dorsal, (c) Polar histogram of MVAs of 89 cells in the left CC. Axis div. = 5 cells. Response of DN VI₂ to lateral stimulation of left (d) and right (e) eyes. Bar 5 Hz. Dotted line = inhibition. A = anterior, P = posterior.

upward movement, one to vertically downward movement Fig. 1a and b). Analysis of the mean vector angles (MVAs) of 89 cells shows a bias towards either the vertical or the horizontal with an asymmetrical distribution within one connective (Fig. 1c). In the left connective more cells sensitive to right to left frontal movement (3) have been identified than cells sensitive to left to right movement, (1). More vertically downward sensitive cells have been identified (4) than upward (2). Cells with the same overall directional sensitivity have differing response characteristics for example, their velocity response curves are different and, in some cases, their response to lateral stimulation. Roll simulation with lateral gratings reveals at least one unit strongly sensitive to downward motion over one eye and weakly sensitive to upward motion over the other eye, (Fig. 1d,e).

Thus amongst the descending units identified there are cells capable of signalling yaw, pitch and roll movements. If and how individual neurons contribute to stability and course control remains to be determined. The contribution of the L_p ocellar neurons must also be considered since at least one of them is sensitive to wide field motion over its adjacent compound eye (Goodman *et al* in press).

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Identified Neck Motoneurons Exhibiting Directional Selectivity to Wide-Field Visual Motion in the Honeybee, *Apis mellifera*

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Worker honeybees exhibit optomotor head turning responses when ventero-lateral regions of the compound eyes are presented with horizontal wide-field moving patterns (Moore *et al.*, 1981). This optomotor response suggests that there is a link between the compound eyes and motoneurons innervating muscles in the neck responsible for head turning. Several descending interneurons originating in the brain exhibit directional selectivity to wide-field visual stimuli and arborise in the suboesophageal and prothoracic ganglia where motoneurons controlling head turning may be expected to originate (Goodman *et al.*, in press). Intracellular recordings were made from a cervical nerve known to terminate in head turning muscles of the neck.

METHODS

Worker honeybees were captured in flight from hives at Queen Mary College, London and briefly anaesthetised with carbon dioxide. The bees were securely restrained and the cervical connectives of the ventral nerve cord were exposed ventrally. The stimulating apparatus consisted of computer-controlled lateral belts and a frontal drum which could present wide-field moving square-wave grating stimuli to the lateral and frontal visual fields of the bee at any orientation in 20° or 7.5° steps. Wind stimuli were provided via a Pasteur pipette directed at the head and body of the bee. Intracellular recordings were made from the cervical nerve IC1 (Markl, 1966) which branches from the cervical connectives of the ventral nerve cord. Cobalt chloride (3M) filled glass capillary microelectrodes (10 to 40 MegOhms) were used to penetrate axons in the cervical nerve. On-line analysis of spike frequency relative to the spontaneous activity of the cell was performed as well as recording to FM tape. Single unit cobalt staining was achieved by passing a pulsed current (50nA) through the electrode after recording. The brain and ventral nerve cord were treated for silver intensification (Bacon and Altman, 1977). The tissue was usually viewed in wholemount from the posterior or side of the brain.

RESULTS

Sections through nerve IC1 have revealed eight axons larger than ten micrometres in diameter. Forty intracellular recordings have shown that there are eight motoneurons in this cervical nerve originating in the suboesophageal ganglion (SOG). The motoneurons are here designated CN1M cells. The motoneurons belong to two anatomically distinct groups of four cells based on the position of cell bodies and arborisations. Figure 1 illustrates the position of the cell body and arborisations of cell CN1M2. Motoneurons CN1M1-4 have cell bodies which lie close to the ventral midline of the SOG. Figure 2 shows the morphology of CN1M7. Motoneurons CN1M5-8 have lateral cell bodies in the SOG.

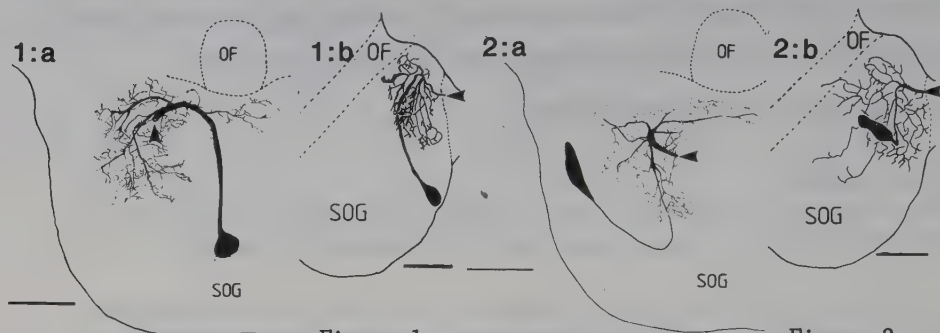


Figure 1.

The morphology of CN1M2.
a) Posterior view of SOG.
b) Side view of SOG. Bar=100um.
OF=Oesophageal foramen.

Figure 2.

The morphology of CN1M7.
a) Posterior view of SOG.
b) Side view of SOG. Bar=100um.
Arrow=position of axon.

Table 1 summarises the physiological responses of the motoneurons to wide-field visual and wind stimuli. When recording from the left cervical nerve and stimulating the frontal visual field, four motoneurons respond to left to right movement, three respond to right to left movement and one responds to vertical downwards movement.

The neck motoneurons described here have responses which suggest that they have roles in optomotor head yaw and pitch. There are also responses to head roll.

CELL NAME	MVA±SD FOR STIMULUS CONTRAST FREQUENCIES			WIND SENSITIVE?
	6Hz	12Hz	20Hz	
CN1M1	83°±55	77°±46	76°±42	NO
CN1M2	—	43°±63	—	NO
CN1M3	269°±52	256°±49	255°±44	YES
CN1M4	189°±54	157°±47	181°±48	YES
CN1M5	148°±74**	73°±61	78°±50	NO
CN1M6	114°±57	100°±53	117°±51	YES
CN1M7	291°±37	289°±47	291°±42	NO
CN1M8	—	56°±66**	—	NO

TABLE 1: Responses of representative cells recorded from the left cervical nerve IC1. MVA±SD=Mean vector angle±standard deviation as calculated for the response of each cell (Batchelet,1981).

**=not significantly directional. —=Not tested.

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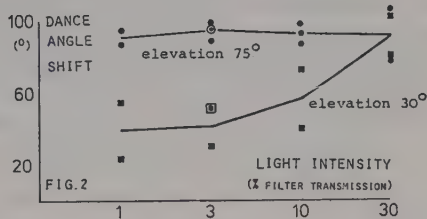
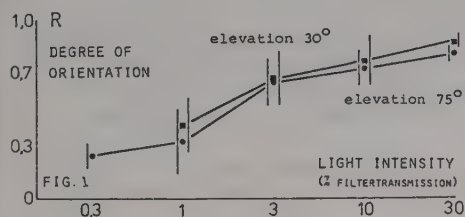
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Polarized Light Orientation in Honey Bees: Additional Data on Functional Specialization in Different Parts of the Bee's Eye

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In experiments with honey bees intensity-threshold measurements of dance orientations were conducted with artificial areas of polarized sky at elevation 75° and 30° above the horizontal. This way we tested the notion that polarized light is evaluated only with ommatidia of the dorsal rim of the eyes (cf. Wehner 1982). While the intensity at each elevation was varied the plane of polarization p was alternatively set at $+45^\circ$ or -45° rel. to vertical. Per stimulus condition 80 waggle angles of forager bees were recorded and the differences established in dance directions ($\Delta\alpha$) for $p +45^\circ$ and -45° . The intensity dependence of the orientedness (in r = length of mean vector) is presented in Fig. 1. The curves for the two elevations indicate comparable sensitivity. The intensity dependence of $\Delta\alpha$ however yielded different curves, Fig. 2. At elevation 30° the effect of polarization is reduced for low intensities and concomitantly the effect of anti-sun orientation is appearin. (Anti-sun orientation is observable with respect to an unpolarized UV light source when bees dance in opposite direction to that under sun or long wave length light; Edrich 1977). Complete predominance of anti-sun behaviour was not observed, then data would have occurred at $\Delta\alpha = 0$.



To summarize: In our experiment the dorsal rim area of the bees eyes appeared primarily responsible for polarized light orientation; while the less dorsal areas contributed to two responses at the same time; to polarized light and to anti-sun orientation. Polarized light evaluation for orientational purposes therefore cannot be limited exclusively to the dorsal rim area of the bees' eyes, a result which is in agreement with earlier findings (Kirschfeld 1972).

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Color Constancy in Bees (*Apis mellifera*)

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Color constancy is the ability to recognize the color of an object independent of the composition of illumination. Chromatic properties and intensity of natural light are always changing; therefore, color constancy is essentially important for the useful functioning of the color vision system, particularly in the case of the honey bee. As E.H.Land demonstrated in his Mondrian-experiments, mans color constancy cannot be explained on the basis of chromatic adaptation, but may be a consequence of the neural mechanism of color coding. According to Lands Retinex-Theory, a suitable process of information in the visual system could be based on a ratio-making principle: the color of an object is determined by the amount of reflected short-, middle- and long-wave-light in contrast to the reflections of all other objects in the visual field.

The aim the experiments introduced here, was to prove whether the color constancy performance of bees is equivalent to that of man, which would then lead to an appropriate principle of color coding in the bee brain.

Materials and Methods

Bees were trained to collect sugar water from one colored plate within a multicolored chessboard arrangement ("Bee-Mondrian"), which was illuminated by short-, middle- and long-wave-light. The set up was similar to that used by E.H.Land used in his Mondrian-experiments.

The experimental principle was:

- 1.to establish the choice-frequency of trained bees under the illumination conditions which prevailed during training (discrimination-tests);
- 2.to change illumination, so that a totally different color plate now possesses the exact chromatic properties of the color plate which had been used during training (color match);
- 3.to determine choice frequency under changed illumination (color-constancy-tests);

Results

Color constancy was investigated in blue-green and violet color regions (4 examples).

In all cases, comparison of discrimination- and color-constancy-tests showed only minimal or no differences in choice-frequencies for the matching colors. So, color vision of bees seems to be relatively independent of illumination properties: color constancy under the experimental conditions of a Mondrian-experiment.

The minimal, but systematic changes in choice behavior indicate,

that there are small shifts in the bees color perception, which are correlated with the direction but never with the total strength of illumination change.

Conclusion

All color areas within the "Bee-Mondrian" were separated from each other by black areas. Therefore, to explain bees performance of color constancy, a mechanism of color coding is concluded, which provides for integration of information over large parts of the visual field. The Retinex-theory of color vision can be successfully applied in interpretation of the results.

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Physiological and Behavioural Characteristics of Sleep in Honey Bees

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Hive observation as well as electrophysiological and behavioral experiments have revealed that, at night, forager honey bees exhibit phenomena which are strongly reminiscent of several features (sleep signs) which accompany sleep in humans and mammals.

Long-term recordings from single, higher-order interneurons of the bee's brain (Kaiser and Steiner-Kaiser, 1983) yielded the first indication that these diurnal insects experience sleep-like states at night. These optomotor neurons display properties surprisingly similar to those shown by interneurons of the visual system of sleeping and waking cats (Livingstone and Hubel, 1981).

Further evidence comes from hive observations at night. Large numbers of worker bees remain still for extended periods of time. These resting bees are located mainly at the outer margins of the combs. Some rest in empty cells (see also Lindauer, 1952). Thermovision measurements showed that resting bees are not actively engaged in heat production. The antennae of resting bees occupy characteristic positions. These positions are also seen in the laboratory in individual, horizontally mounted bees walking on a treadmill (Fig. 1). In these animals, a reduction in neck-muscle tone can be regularly observed - the inclination of the head gradually increases in the course of the night and the amplitude of the electromyogram of neck muscle 42 declines progressively. Minimum muscle tone occurs late at night.

Stimulation of the antennae of individual, fixed foragers with pulses of infrared radiation elicits grooming movements. The reaction threshold for this response is much higher at night than during the day. Highest threshold values occur late at night (Kaiser, 1985).

Individual, unrestrained foragers were placed in a lucite chamber and observed continuously with infrared-sensitive video equipment. Such bees remain in one location and display reduced motility of body and appendages for long periods of time (7-9 h) at night.

Numerous descriptions of "sleeping" insects, particularly Hymenoptera, were published in the first half of this century (e.g. Fiebrig, 1912, Rau and Rau, 1916). The results reported here (see Kaiser, in press, for details) are the first investigations of physiological changes associated with the nightly resting state of honey bees. The many similarities between sleep in humans and higher vertebrates and the phenomena displayed by resting bees (raised reaction threshold, decreased muscle tone, motility and body temperature and reduced neuronal sensitivity) strongly support the hypothesis that sleep occurs in bees, too. Thus we hope that further investigation of sleep in honey bees, particularly at the cellular level, will reveal general principles common to a wide variety of animals including mammals.

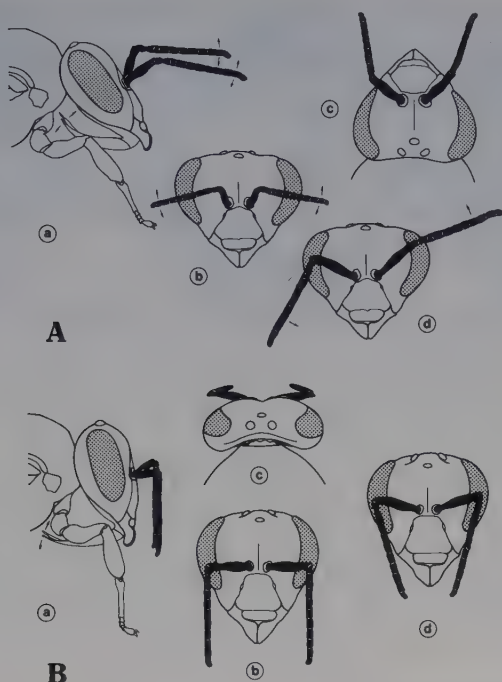


Fig. 1. -- Drawings traced from the screen of an infrared-sensitive video system. Side, frontal and top views [(a), (b), (c), respectively] were recorded simultaneously with the aid of surface mirrors. The (d)-views were obtained at other times from the same animal. The antennal positions in A typically occur during locomotor activity in the daytime, the positions in B when bees remain very still at night. B(d) was always associated with even higher reaction thresholds than B(b). The transition from A to B is gradual.

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Morphofunctional Comparative Study of the Spatial Organization of the Sensory Antennal Pathway in the Workerbee, the Drone and the Queen

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This work takes place within the general context of our research concerning the functioning, the ontogeny and the plasticity of the olfactory system in the honeybee (Arnold and Masson, 1983 ; Masson and Arnold, 1984 ; Gascuel et al., this issue). This study involves a precise knowledge of the hodologic organization of the antennal lobe, at the level of the synaptic fields of association between the first neurones (antennal sensory cells) and the second neurones, in the glomeruli

The majority of antennal sensory axons project to the antennal lobe of the deutocerebrum where they converge onto the dendrites of several thousand deutoneurons in dense structures of neuropile : the deutocerebral glomeruli, at which level the synaptic connections are localized (Schürmann and Wechsler, 1970 ; Masson, 1977). The deutocerebral glomeruli can be quantified morphologically (Chambille et al., 1980).

MATERIALS AND METHODS

The topography of the afferent antennal pathway analysed by cellular marking following localized application with cobalt chloride to the cut end of one antenna. The serial sections (7µm) were stained on slides according to the TIMM method. 110 workerbees, 45 drones and 21 queens were used for labelling. Of these, the counting and measurements of dimensions of all glomeruli were done in 5 workerbees, 5 drones and 3 queens (Arnold et al., 1985, 1986).

RESULTS

In the 3 castes, 4 main regions of the glomerular neuropile have been distinguished according to corresponding afferent bundles. The pathway of these bundles are very similar, but the diameter of the T1 bundle is bigger in the drone and smaller in the queen, due respectively to the larger number and the smaller number of the sensory cells in the antenna (Fig. 1).

In the 3 castes, the majority of the glomeruli (95 %) had a characteristic structure involving a cortical layer (dyed intense black with cobalt) at which level the synaptic connections between the sensory axons and the deutoneurons take place. A small number of glomeruli (7), located in the posterior region had a cortical layer little or not at all differentiated which seems to suggest functional differences for these glomeruli.

The organization of the antennal afferent pathway of the queen is close to the worker's. The main difference is the existence of a glomerulus which volume is relatively more important in the queen and which could be a macroglomerulus. An important sexual dimorphism, represented by 4 large and easily identifiable glomerular complexes, was demonstrated in the drone. In other insects such as moths and cockroaches, the hypertrophied glomeruli, glomerular complex or macroglomerulus, receive only sexual information.



Fig. 1. Comparative frontal sections of one antennal lobe of a worker bee (A), a queen (B) and a drone (C).

(G:glomeruli, GC:glomerular complex, MG:macroglomerulus, $\bar{\perp}$:50 μ m)

The number, position and dimensions of the glomeruli indicated that the glomerular organization was unvarying in each caste.

Despite slight differences the trail and the projection areas of deutocerebral efferences were very similar in the worker bee, the queen and the drone. The efferent axons leaving the antennal lobe made their way towards the ipsilateral protocerebrum by way of 4 bundles which project into the calyces of the mushroom bodies and the lateral lobe of the protocerebrum.

Our results concerning overall invariance of the organization of the antennal lobe in the 3 castes, together with the presence of several types of glomeruli support the hypothesis of the functional unit of the glomeruli (Masson, 1973). Functional and immunocytochemical investigations are presently in progress to confirm this hypothesis (Arnold et al., this issue).

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Comparative Neurobiological and Chemical Data on Communication in Bumblebee Males and Females

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Chemical senses are essential sensory channels in bumblebees (*Bombus* sp.) : the main relationships between the members of the colony rely on chemical signals such as pheromones. Besides plant allelochemicals are used in the foraging behaviour (Fonta et al., 1985). Different molecules, detected at the peripheral nervous system (P.N.S.) level and integrated in the central nervous systems (C.N.S.), may thus trigger or release various behaviours by males, queens and workers.

We have first studied the detection equipment and functioning, then the central organization of the olfactory nervous system in both sexes and castes of 2 bumblebee species, *B. hypnorum* and *B. terrestris*. In parallel labial gland secretions and cuticular extracts have been analysed in *B. hypnorum*.

NEUROBIOLOGICAL DATA

P.N.S. : The population of s.placodea, antennal olfactory sensory organs, were counted on each flagellum article. The average density is comparable in queens and workers ($\approx 3760/\text{mm}^2$) ; it is weaker in males ($\approx 3370/\text{mm}^2$).

Assessment of the total number of these sensilla shows that global differences between worker, queen and male might be induced by the insect size variation (Fonta and Masson, 1982).

EAG recordings in response to pure odour stimulations (floral and pheromonal compounds) have always higher amplitudes for queens. Amplitudes in workers and males are comparable. Among the tested substances, farnesol (derivative of a pheromonal component) and vanillin (usual floral aroma compound) are detected with a high sensitivity (Fonta and Masson, 1984).

C.N.S. : The central projection of antennal fibers was studied by using a method of cellular staining with Co++ ions.

A striking difference in the anterior region of the antennal lobe exists between males and females : 3 macroglomeruli receiving inputs by 2 different bundles of the antennal nerve are present in the male (Fonta and Masson, 1985).

CHEMICAL DATA

Extracts in hexane of cephalic labial glands and cuticles were analyzed by GC-MS (Genin et al., 1984). Male labial glands are known to produce the marking pheromone (Kullenberg et al., 1973) used for mating. Van Honk et al. (1978) suggest that cephalic secretions of the young queens rule the copulation.

Qualitative spectra of cuticular and glandular extracts are different for male, queen and worker ; e.g. fatty alcohols, fatty acids discriminate males and females; esters of geranylgeraniol characterize the queen ; geranycitronellol and esters of geranycitronellyl are main components in the worker extracts.

Besides the quantitative variations in individual spectra depends on the physiological state of the insects (relative ratios of diterpenic alcohols and of their esters vary between virgin and mated queens).

CONCLUSION

The olfactory PNS shows some structural and functional similarities between males and females. A central sexual dimorphism suggests that an integration pathway favours chemical sexual inputs in the male brain.

The insect secretions are complex mixtures qualitatively characteristic of each sex and each female caste. Their quantitative variations might be cues for the temporal evolution in biological and physiological parameters of the bumblebees.

Further pluridisciplinary approaches are now necessary to determine at which level of the neuronal network sexual and caste specificities are implied in the spatial and temporal patterns of precise olfactory messages processing.

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Mechanism of Hypopharyngeal Gland Activation in Honey Bees (*Apis mellifera*)

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Honey bee larvae depend completely on adult worker bees for their food. The proteinaceous component of the food for the first three days of larval development is supplied by the hypopharyngeal (HP) gland (Hoffmann, *Ztschr. F. Bienenforsch.* 8:296-322). When brood is absent, protein synthesis remains at a low level, but it can be activated to a maximum within 3 days in the presence of brood (Brouwers, *J. apic. Res.* 22:137-141). Together with other evidence, we suspect that honey bee brood may emit a signal to activate the protein synthesis of HP glands, and thus ensure their food supply. This study investigated the property of the signal and its mechanism of gland activation.

A modified Brouwers (*J. apic. Res.* 21:193-198) *in vitro* method (first experiment) and an *in vivo* method (all subsequent experiments) were used to measure HP gland activity. In both methods, the amount of radioactive leucine absorbed into the glands indicated the synthetic activity of the glands.

In the first experiment, bees 8 days old were sampled from the broodless and "brood-right" sides of colonies, divided either by a single screen or a double screen, and from completely broodless colonies. Bees from brood-right situations had significantly higher HP gland activity than broodless portions. However, the HP gland activity of broodless bees separated from brood by single or double screen was not significantly higher than that of completely broodless bees.

The second experiment was designed to see which brood stage is responsible for the gland activation. Colonies were first made broodless by caging the queen. Then into each of 5 colonies was introduced a single frame with only one stage of brood: eggs, larvae, capped brood, or a mixture of all the three stages. Bees were then sampled from those frames to determine HP gland activity. It was found that only larvae or the mixture of all brood stages activated the HP glands.

To determine if the signal is a chemical, we also prepared surface washes and whole body extracts in various solvents. None of these extracts, whether obtained by ether, acetone, hexane or water, or applied to filter paper, comb, rubber septa or sugar syrup, had any stimulating effect on the gland activity. Neither did living larvae presented to small groups of bees on filter papers.

In conclusion, the result of our first experiment indicated that the signal from the brood was not a volatile chemical, nor something transferrable through trophallaxis, but a stimulus which must be directly contacted by worker bees. The second experiment showed that the signal was only possessed by the larval stage, not by eggs or capped brood. Extracts in various solvents failed to activate the HP glands of bees kept in small cages. Therefore either 1) the signal is not chemical or 2) the signal is chemical but needs other co-factors or experimental conditions to be effective.

Communication by Infrasonic Pulsations in the Honey Bee

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Prolonged monitoring of changes in haemolymph pressure by means of sensitive electronic methods (Sláma, 1984) have revealed that the metamorphosis stages (prepupae and pupae) of the honey bee exhibit genuine infrasonic pulsations. The common measures of these extracardiac pulsations in pupae of various castes are as follows: duration 5-20 min., resting periods between pulses 10-80 min., frequency 0.1-1 Hz, subatmospheric baseline haemolymph pressure -5 to -20 Pa (related to atmospheric zero), maximum amplitude of the pulses 20-50 Pa. The measures show characteristic and progressive changes which are related to the progress of adult development. Thus, the young pupae of all castes exhibit different patterns of pulsations than a more advanced stage and a pigmented pharate adult shows almost continuous pulsations of several types. In other insects the extracardiac pulsations appear regulated by an autonomic, brain-independent, parasympathetic-like nerve system (Sláma, 1986), which is susceptible to slight changes in internal water content or in the ambient temperature and humidity.

The young worker bees can recognize and incubate healthy brood in the closed cells. According to Koeniger (1984), this recognition is not based on a simple mechanical interaction, as the dead pupae or objects of similar weight are not incubated. Zdarek *et al.* (1976) observed selective removal of the living pupal brood with the faulty development that had been caused by analogues of juvenile hormone. The selective removal of the specimens with defective development could hardly be explained by purely chemical interactions. We believe (and experiments are on the way to prove it) that worker bees can perceive mechanically the extracardiac pulsations of individual pupae in the closed brood. In this way they communicate with the brood, supervise and check the developmental progress by monitoring the extant type of the infrasonic pulsations.

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Evidence for a Sensitive Period during the Development of the Olfactory System in Honey Bees: Anatomical, Functional and Behavioural Data

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In order to define the limits of worker bees olfactory plasticity, we carried out a multidisciplinary approach to specify the role of epigenetic factors in the setting up and the functional stabilization of deutocerebral neuronal networks and their behavioural outcomes. In honey bee the antennal neurons project into the antennal lobe of the deutocerebrum (DTC) where they converge onto output neurons and local interneurons (gabaergic inhibitory neurons in majority (1). This convergence takes place in deutocerebral glomeruli. The synaptic connections are strictly localised in cortical layers (2).

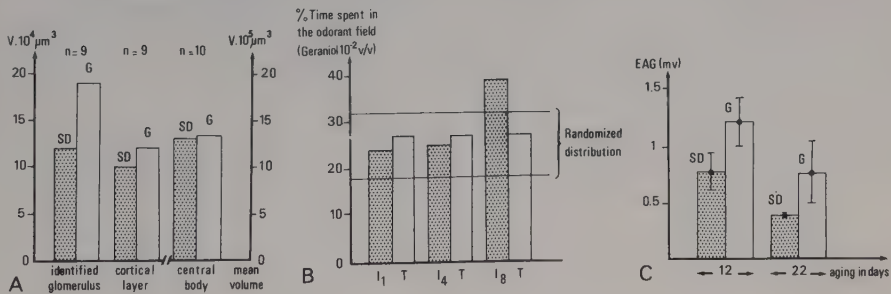
1 - Data suggesting a "sensitive period". Anatomical, electrophysiological and behavioural data allowed to point out a period of maturation during which worker bee development may be particularly sensitive to environmental changes. (i) At structural level, during the ontogeny the first synapses between afferent olfactory neurons and deutoneurons take place as soon as 7 days before emergence (E-7), but adult-like structure of the antennal lobe with a typical cortical layer of individualised glomeruli only appears at E-3 (3). These data suggest that the rapid and extensive development of the final organisation of the synaptic fields in the DTC could be closely related to the huge increase in numbers of local interneurons involved in data processing of the huge antennal informational flow. (ii) At physiological level, classical EAG recordings point out a maturation of antennal responses to olfactory stimuli during 4-6 days following the emergence day. Moreover emerging bees already perform a level of 30 % EAG responses compared to 4-6 days old bees, which suggests that olfactory system may be functional before the emergence. Recent experiments demonstrate that this maturation begins circa 2-3 days before emergence day. (iii) At behavioural level, observations of the courtship behaviour of worker bees towards queen pheromone extract show that the highest level of attraction is obtained to 1 to 6 days old worker bees.

2 - Experimental approach : deprivation and imprinting. In order to specify if the period of time between circa 3 days before the emergence day and 6 days after is a sensitive period, neuroanatomical, electrophysiological, and behavioural studies have been carried out, after controlled modifications of honey bee olfactory environment. The biological material used has a controlled genetic homogeneity through artificial insemination of the queen with one drone. (i) Effect of deprivation onto the stabilization of glomerular synapses and peripheral functioning. Social deprived bees are reared alone from the first day of their pupal stage, while normal grouped bees used as controls are maintained, out of the hive, in the odour colony environment. Quantitative analysis through semi-automatic measurement of serial histological sections of cortical layer and glomeruli is carried out after cellular staining by cobalt ions migration on

antennal cut end of deprived bees (SD) and controls (G.). It appears that such a social deprivation induces a decrease in the volume both of identified glomeruli associated with specific antennal nerve bundle T1 and their cortical layers, while the volume of the central body is not affected (Fig. A). That suggests that the DTC level is drastically affected by epigenetic factors. Moreover electrophysiological responses to butanol are recorded on 12 and 22 days old worker bees deprived during 6 days from emergence. The isolated bees are subsequently always less responsive by 20-30 % than the bees reared in normal conditions (Fig. C). (ii) Effect of imprinting onto adult olfactory behaviour. A biotest based on an orientation behaviour in a dynamic flow choices olfactometer is applied to samples of 12 days old worker bees previously maintained in a high scented environment (Geraniol). Olfactory choices are recorded for different imprinting durations (1-4-8 days) (I), and compared to naives bees (T). No differences arise for 1 and 4 days of imprinting while 8 days imprinted bees exhibit a significant preference for the imprinting scent (Fig. B).

CONCLUSIONS

From now on, these convergent data allowed to specify the hypothesis of a sensitive period, lasting from 3 days before emergence to 6-8 days of adult life, where olfactory experience is decisive in the future functioning of honey bee olfactory system and final stabilization of synaptic connections. Currently, studies are in progress both at structural and behavioural levels. They might allow to estimate synaptic qualitative and quantitative changes after epigenetic factors modification and to define the type of synapses (excitatory and/or inhibitory) affected and to specify the exact limits of imprinting phenomenon.



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2.3 Thermoregulation

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The Thermoelectric Properties of the Integument in Social Wasps

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The following physical characteristics have been detected in the Vespinae integument measured by two electrodes distanced 5 mm apart:

1) D.C. resistance values of 10^7 to 10^8 ohm generally with a negative temperature coefficient, photosensitivity with traps, these effects indicating the presence of semiconductive properties: the brown colored cuticle being of the n type, while the yellow colored is of the p type conduction, thermoelectric (Seebeck) effect; 2) electrical capacitance around 3.0 nF; 3) piezoelectric properties; 4) These electrical properties, which are age and temperature dependent at any point measured on the integument are repeatable.

The possible use of these electrical properties in energy transfer, in sensing and regulating the ambience and in filtering and synchronizing signals with nest mates is discussed.

MATERIALS AND METHODS

Specimen collection and preparation was performed in the same manner as described by Ishay, 1964; Ishay and Sadeh, 1975; Ishay and Shimony, 1982. The various resistance temperature coefficient measurements and the influence of feeding colchicine and purine on hornets were described by Ishay et al, 1982a,b. The effects of xanthines (theophylline and allopurinol) are described by Rosenzweig et al (in press). A comparison of the temperature coefficients measured on various Vespinae species was made by Ishay et al, 1982c, and a statistical model formulated by Ishay et al, 1985. The photo-sensitive properties were previously described by Croitoru et al, 1978; Ishay and Croitoru, 1978; the venom effect by Ishay et al, 1979, and a mathematical model by Ishay et al, 1980; thermoelectric and capacitance measurements by Shimony and Ishay, 1981, and 1984, respectively. Mechanical disturbance of the integument produces corresponding voltage outputs and a reproducible change in the polarity sign (Ishay, unpublished).

RESULTS

The resistance to D.C. current of Vespinae integument is in the range of 10^7 - 10^8 ohm and of 10^6 - 10^7 to A.C. at 50 hz. When measured between two electrodes at a distance of 5 mm the general trend is as follows: there is a drastic decline in resistance from -35°C to about $+26^\circ\text{C}$, then a plateau with almost no changes in resistance with an increase in temperature and, finally, a rise in resistance up to 45°C (the highest temperature measured). Between the curves for increasing and decreasing temperature a hysteresis forms, i.e., the warming and cooling curves do not coincide or overlap. Feeding on colchicine and purine changes the resistance values, while feeding on xanthines 'straightens up' the cooling vs warming curves.

The cuticle exhibits photosensitivity upon irradiation with light, the effect of which is reversible. Spraying venom on the integument damages the photosensitive properties. In living as well as in dead specimens measured, the resistance first increases until it reaches a 'limit', acting like an organic photoresistor (Inokuchi and Akamatu, 1961) with traps. The 'limit' is attained only when irradiated for 15-20 min with integral white light, while irradiation with the light of a He-Ne laser produces the limit level almost immediately. After attaining the 'limit' line the resistance decreases by 10-15% under light, but returned almost to the initial value in the dark.

The thermoelectric (Seebeck) coefficients ($S = \Delta V / \Delta T$) in various cuticular areas of *V. orientalis* varies from 0.3 to 2.4 mVdeg⁻¹, within a temperature range of 27-36°C when the temperature difference between the two measuring electrodes (ΔT) varied from 0.6-8.0°C. The signs of the effects measured on the brown colored cuticle suggest an n(-) type conduction while those measured on the yellow stripes point to a p(+) type conduction. The hornet integument has an electric capacitance of around 3.0 nF.

The electrical changes obtained by applying pressure to the integument suggests the presence of piezoelectric properties in the range of 100-300 mV.

DISCUSSION

Electron microscopy has revealed the presence of many laminated layers in the integument, both in the yellow as well as in the brown stripes (Neville, 1975; Ishay et al, in press). The D.C. resistance was exhibited between electrodes placed in the plane of the outer laminate.

The tanning process enables the cuticle to act as a semiconductor (Digby, 1965) since there is an alternation of single and double covalent bands in the aromatic rings of the tanning agents, among others, quinons (Ikan et al, 1968) and presumably, also in melanin the so-called 'low molecular conjugated system'. The many layers of aromatic rings are held in a state of congruence by magnetic interaction and overlapping of the π orbitals of the neighbor rings. Gaps between such aromatic rings are crossed by a current of excited electrons. The π electrons arranged perpendicularly to the plane of the rings.

Upon the initiation of heat, more and more electrons are excited, so that the resistance diminishes with a rise in the temperature, as per the typical Arrhenius pattern. As the temperature continues to rise, the layers commence moving one against the other, which hampers the transport of electrons since the mean distance between the rings increases. At the plateau stage, there is still a balance between the two factors, namely, the number of charged electrons and the ability to pass 'between' the rings. However, with a further temperature rise, the layer vibration receives prominence so that despite the increase in the number of charge carriers, the conductivity diminishes owing to the second factor. The presence of dipoles of water molecules acts to consolidate the ties between the rings (creating a locker effect), i.e. a rise in the humidity, should result in an increased conductivity. However, at temperatures beyond 30°C, there is marked evaporation (Neville, 1975) and, consequently, a sharp rise in the resistance.

The pigment in the brown integument is melanin, known as a typical amorphous n-type semiconductor with free radicals (Ishay and Shimony, 1983; Proctor, 1986) exhibiting such properties as threshold and memory switching (McGinness et al, 1974). A fundamental characteristic of an amorphous semiconductor is strong phonon-photon coupling, i.e., energy is readily transferred between phonons (e.g. molecular vibration heat and sound) and photons (electronic vibrations), i.e., melanin efficiently converts light into heat - an electron-phonon conversion or, in the reverse direction, converts vibrational energy to electronic energy, making melanin the best sound absorbing material known. Melanin even makes a fairly good battery (McGinness, 1982) and can function as an efficient superoxide dismutase (Geremia et al, 1984; Proctor and Reynolds, 1984). Electron excess in the brown stripes may be transferred to or from the yellow pigment, supposedly containing crystals of purines and pteridines in social wasps while containing other pigments in butterflies (Ziegler and Harmsen, 1969; Rembold et al 1978). Both behave like a photo-conductor with traps: the electron receiving enough light energy (photons) are transferred from the basic level (valence band) to a higher level (conduction band) where some are trapped to O_2 and form a complex O_2^+ which is rich in energy, the energy received in the photochemical reaction (Kustanovitch et al., 1964; Patalach et al., 1964). These traps can be separated when supplied with thermal energy, at which point the liberated electron can participate in biochemical reactions.

The piezoelectric effect could be responsible for many phenomena present in these insects, such as transduction of energy (pressure to current and vice-versa), that are connected to sensing changes in the ambience (light, temp., gravity, sounds) and adjust accordingly, as well as signals from the nest mates.

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Thermoregulation by Individual Honeybees

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Few aspects of insect thermoregulation have been so long and so well explored as that of honeybees. But the physiology and behavior of thermoregulation by individual bees has not been consolidated. I here provide an overview of our present knowledge of thermoregulation by individual bees and point out puzzles that remain to be elucidated.

I. HEAT PRODUCTION

Warm-up. Honeybees produce heat in the thorax prior to flight by contractions of the flight muscles, and the increase in muscle temperature is correlated with action potentials. External vibrations of the thorax are often not measurable during heat production (Esch, 1964), although the dorso-ventral (DV) flight muscles shorten with individual twitches in response to excitation during heat production prior to flight (Esch and Bastian, 1968; Bastian and Esch, 1970). The electrical activity of the dorsal longitudinal (DL) muscles is apparently no different than that of the DV. During warm-up the two sets of activated muscles stretch against each other to produce isotonic contraction, and a stop in electrical activity releases the tension and heat production stops (Esch and Bastian, 1968).

Flight. The action potential specific rate of oxygen consumption (and heat production) is constant between warm-up and flight (Bastian and Esch, 1970), and since both the frequency and shape of the action potentials are similar during warm-up and during flight (Esch and Bastian, 1968) it is of interest to determine why the muscles are used to produce isotonic contractions on the one hand, while alternating their contractions to move the wings on the other. The action potential frequency (and associated muscle tension) could not in itself be the reason for the switch-over from the non-oscillating mode during warm-up to the oscillating during flight. Esch and Bastian (1968) suggest that the momentary high action potential frequency of both the DV and DL muscles prior to flight is the command to start the oscillations. However, the role of the direct flight muscles and the role of the articulations of the wings that would allow them to beat, and hence allow the alternate contractions and stretching of the indirect muscles, is so far not well understood.

Aerodynamic power (and heat production) of the honeybee flight system is positively correlated with action potential frequencies of the indirect flight muscles (Esch, Nachtigall and Kogge, 1975). However, with increasing muscle temperature wingbeat frequencies can increase from 120 Hz to 190 Hz, lift can more than double, and flight

velocity triple, while the action potential frequency does not change significantly (Esch, 1976). The large increase in power output under constant neural activation is likely due to increases of efficiency of the flight muscles when they are at a higher temperature.

Before the significance of thermoregulation was understood, an insect's stopping in flight was often interpreted as an indication of fatigue. For example, honeybees need more time to return from a feeder than for the flight out to it, and von Frisch (1967) supposed "That is connected with the fact that with their burden they do not infrequently stop to rest." If stopping in flight is related to thermoregulation, however, then, it can be predicted that return time would shorten at higher ambient temperatures (T_a).

Honeybees have a preferred body temperature near 35°C (Cahill and Lustick, 1976), and bees within the core of a swarm cluster, within the brood nest, and around freshly built honeycomb maintain a body temperature within 1°C of 35°C (Himmer, 1927; Heinrich 1981). The thermal stability of the large mass of thousands of bees is a product of the combined endothermy of the individuals. However, individuals by themselves who are not in flight show a highly variable thoracic temperature (T_{th}), with T_{th} often fluctuating 10°C over 2 minutes or less (Heinrich, 1979), presumably due to on-off shivering (Bastian and Esch, 1970).

Individual bees within the hive, such as drones (Cahill and Lustick, 1976), could potentially be passive with regard to thermoregulation and still experience near optimal body temperatures. However, if all bees adopted the same passive strategy, then sub-optimum hive temperatures might result. Very little is understood relative to what extent individuals attempt to regulate hive temperature, and to what extent they regulate their own body temperature and then secondarily affect hive temperature. Considerable, but by no means all, of the observed thermoregulation of honeybee swarms can be explained in terms of bees regulating their own body temperatures (Heinrich, 1986), while other data show that individual bees are willing to put themselves in thermal stress to help regulate hive temperature (Vogt and Heinrich, 1985).

II. HEAT LOSS

The circulatory system. The aorta in honeybees, unlike that in wasps, hornets, leafcutter bees and bumblebees, is highly convoluted in the petiole area. It has 9 loops attached to each other and enclosed in a membrane (Freudenstein 1928). One possibility is that the loops function in thermoregulation. According to Freudenstein (1928) blood from the thorax flows over and around the loops before returning to the abdomen. Since the honeybee's thorax is heated by endogenous heat production, this blood is necessarily hot. On the other hand, the blood flowing through the coils from the unheated abdomen (Heinrich, 1986) is necessarily cool. The coils could function in three ways to promote counter-current heat exchange. First, they would obliterate discrete pulses of blood that could be quickly shuttled through the petiole, as occurs in bumblebees when they dump heat into the abdomen (Heinrich, 1976). Secondly, the loops would create a large surface area for possible counter-current heat exchange. Lastly, they would provide resistance and slow down the blood so that there is more time for counter-current heat exchange.

As predicted by the above hypothesis, honeybees do not transfer excess heat from the thorax to the abdomen (Heinrich, 1980b). However, the carpenter bees, *Xylocopa varipuncta*, which also have a series of aortal loops in the petiole area, transfer considerable amounts of heat to the abdomen from the thorax (Heinrich and Buchmann, 1986).

Evaporative cooling. Lindauer (1954) showed that a colony of honeybees can cool itself by evaporative cooling. During colony over-heating the hive bees selectively accept dilute nectar or water and the previously preferred foraging for concentrated sugar solutions changes to water collecting. Individual bees regurgitate dilute nectar, or water, hold it on the tongue, and deposit it on the combs. Esch (1976) observed that bees in flight at high air temperatures also regurgitate fluid from the honeycrop, and they work it with their mouthparts and sometimes spread it over the underside of their thorax with their legs, presumably for evaporative cooling.

Laboratory experiments (Heinrich, 1980a+b) directly confirmed the hypothesis that honeybee workers stabilize thoracic temperature during flight by evaporative cooling using regurgitated honeycrop contents.

The ability to dissipate heat from the thorax is due primarily to evaporative cooling from the head. Bees that can fly at $T_a > 40^\circ\text{C}$ extrude droplets of fluid at the mouth, and their head temperatures is on the average 2°C below T_a (Heinrich, 1980a). Evaporative cooling from the tracheal system has been briefly investigated (Heinrich, 1980b) and so far its significance has not been shown to be of primary importance. Head temperature closely tracks T_{th} due to conductive heat flow (Heinrich, 1980a), although the circulatory system probably aids in heat transfer.

The physiological model of thermoregulation in honeybees that emerges is that head temperature is regulated by defending an upper set-point. Thoracic temperature is tightly coupled to head temperature, and T_{th} is maintained $< 46^\circ\text{C}$ secondarily, since heating of the thorax to near lethal temperatures by itself elicits neither the regurgitation of cooling liquid nor the associated response of the aorta. The thorax on the other hand contains a lower set-point (near 36°C) below which shivering occurs. At these (lower) temperatures head temperature is a passive function of T_{th} .

Cooper et al. (1985) confirmed that honeybees regulate head and thoracic temperature at high T_a by regurgitating droplets of fluid that are then evaporated. Furthermore, they extended the observations to provide an ecological perspective of honeybees foraging in the Sonoran desert at high air temperatures. In the desert bees forage for nectar (but not pollen) at T_a above 40°C , and the proportion of bees returning to the hive who carry a fluid droplet on the tongue increases from essentially none at 20°C to 40% at 40°C . Pollen foragers, who on the average carry only $1.3\ \mu\text{l}$ of fluid ($7.9\ \mu\text{l}$ in other bees) decrease their activity at high T_a , but those pollen foragers who forage at high T_a almost never regurgitate fluid, and they have significantly higher T_{th} than the nectar foragers at the same T_a (Cooper et al., 1985). Some of the pollen foragers may have T_{th} near 50°C , their lethal limit.

A heat budget of foraging bees reveals that bees could fly for 45 s at $T_a = 40^\circ\text{C}$ before T_{th} would increase from 40 to 50°C , but evaporation of $1.58\ \text{mg}\ \text{H}_2\text{O}$ over the 45 s flight would prevent any increase in body temperature.

Both heat production and heat loss mechanisms have been examined in terms of physiology and in their ecological contexts, and I conclude that there is probably more known about the thermoregulatory physiology of honeybees than about any other insect. However, numerous questions remain to be explored.

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FREE COMMUNICATIONS

Regulation of Respiration in Honey Bee Colonies

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In the perennial social colonies of honey bees (*Apis mellifera* L.) the nest environment is partially controlled by ventilation resulting from fanning behavior of groups of workers. I report here on the influence of temperature and light direction on the fanning response and ventilation.

METHODS

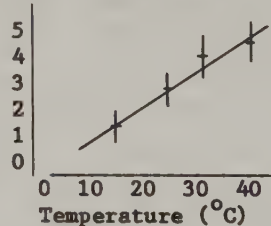
Ten colonies, each with a queen, brood and 2000 workers, were tested in an acrylic nest chamber (15x20x30 cm) under dark conditions. This chamber had a single bottom entrance and was instrumented for air flow determinations. Temperatures, and carbon dioxide and oxygen gas concentrations were continuously monitored during tests conducted at outside air temperatures of 15, 25, 33 and 40°C.

RESULTS AND DISCUSSION

Fanning behavior inside and outside the nest entrance was coordinated so as to create an outflow convection current of warm stale air followed by an inflow of fresh cool air in a regular tidal pattern. The average ventilatory frequency was 2.7 times per min with air exchange rates averaging 0.4 liter/min. At warm temperatures, legions of fanners occupied positions on inner surfaces and always fanned toward light regardless of their locations relative to the nest entrance. More workers fanned at higher temperatures. However, the position of the few fanners at the entrance was the critical factor influencing direction of air flow into or out of the nest cavity. As air temperature outside the nest increased from 15 to 40°C, the ventilation increased following the relation:

$$V = 1.26T - 5.27 \quad (r=0.66, n=55) \quad \left(\Delta \frac{\%O_2}{V} \right)$$

where V is rate of ventilation (indicated by the amplitude of the change in oxygen content at the nest entrance)
T is the outside air temperature



This shows a doubling of the rate of ventilation when air temperature rises from 20 to 40°C which corresponds to a 30% increase in the rate of metabolism to accomplish the work (Southwick, E.E. 1982. Comp. Biochem. Physiol. 71A:277). Good ventilation is assured by the coordinated fanning behavior of workers, especially those located at the entrance.

ACKNOWLEDGEMENTS

This study was done in cooperation with Dr. R.F.A. Moritz who was supported by a Feodor Lynen Fellowship at State University of New York.

Effect of Roxion-S (Dimethoate) on the Body Temperature of the Honey-Bee

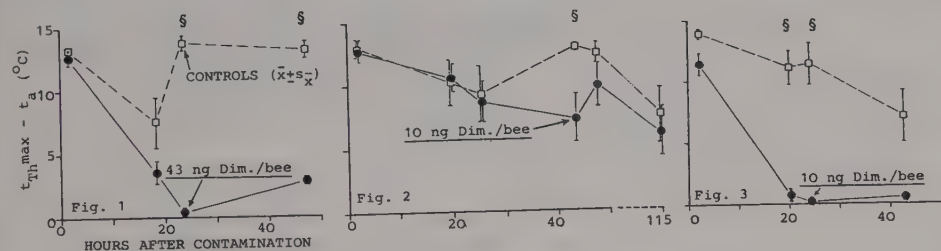
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For the evaluation of sublethal effects of insecticides on *Apis mellifera carnica* POLLM. the body temperature was taken as a bioindicator.

METHODS: Foragers were after 1 hour of stravation individually fed with 30 μ l 1M sucrose-solution, which was contaminated with various amounts of Roxion-S (Celamerck, 400 g Dimethoate/4l Roxion-S). Then they were kept isolated in "Liebefelder-boxes" with 1M sucrose-solution available. In each experiment the surface temperatures of 10 contaminated bees and 10 controls were measured by an AGA 782 SW infrared scanner through an IR-transmissive foil. For thermographic measurements on bees see SCHMARANZER 1983, 1984; STABENTHEINER & SCHMARANZER 1986. The band-emissivity (3,5-5,6 μ m) of the heated intact thorax is $0,99 \pm 0,028(s)$ (STABENTHEINER & SCHMARANZER in prep.). Absolute measurement accuracy: $< + 0,35$ $^{\circ}C$.

RESULTS: The highest thoracic temperatures ($t_{Th,max}$) reached by poisoned bees and controls within the observation period of 2 min (referred to the ambient temperature $t_a = 23,0-26,5$ $^{\circ}C$) were compared. Already 5 hours after contamination with 70 ng Dimethoate/bee the treated animals had up to 16 $^{\circ}C$ lower mean $t_{Th,max}$. The animals fed sublethally with 43 ng Dim./bee showed up to 13 $^{\circ}C$ lower mean $t_{Th,max}$ (Fig.1-3, §: $P < 0,02$, U-test).



DISCUSSION: The sublethally poisoned bees were strongly irritated in their thermal behaviour. Their cooler thoraxes indicate reduced flight muscle activity and thus an abnormal diminished metabolic rate. Body temperature could be shown to be a very sensitive parameter for detection of disease in intoxicated honeybees.

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Influence of Temperature and Seasonality on the Respiratory Metabolism of Castes of Tropical Wasps (*Polistes simillimus* Zikán, 1951)

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Machado (1983) has described colonies of *Polistes simillimus* forming winter aggregations of males, workers and queens, which have well developed fatty bodies and considerably reduced activity levels.

This winter aggregation suggests a rudimentary diapause, as an adaptation to environmental conditions. The respiratory metabolism ($\dot{V}O_2 = \mu\text{l O}_2 \text{ mg}^{-1} \text{ hour}^{-1}$) of queens and workers of *P. simillimus* was studied, in the winter and summer, at different temperatures, to detect the possibilities of metabolic adjustments to temperature changes and to seasonal acclimatization.

MATERIAL AND METHODS

Nests of *P. simillimus* were collected in Rio Claro, SP., Brazil (22° 25' S, 47° 32' W), from December to March (summer) and from June to August (winter) and maintained in a climatic chamber at 25°C for at least 24 hours before the beginning of an experiment. Measurements of the oxygen consumption were made in a Warburg respirometer at 10, 15, 20, 25, 30 and 35°C, using 10 wasps for each temperature experiment. The castes were identified through insect dissection after finishing the experiments.

RESULTS

The respiratory metabolism of workers and queens, maintained individually in the Warburg flasks, increased with temperature and did not show a short-term metabolic compensation.

The statistical analysis revealed that there was no significant difference in intensity of respiratory rate of *P. simillimus* in winter and summer, at various experimental temperatures nor in interactions between castes, temperatures and seasons of the year. However, there was a significant difference between the respiratory rate of workers and queens, which was more evident during the summer. This result is possibly related to a more intense activity egg maturation and oviposition at this season of the year.

The temperature coefficients (Q_{10} values), based on average respiratory rates, were higher at low temperatures and reached minimum values between 20 and 30°C in the winter and 25 and 35°C in the summer.

The lower values of Q_{10} at higher temperatures reflect the thermal ranges of better physiological adjustment during the winter and summer for queens and workers of *P. simillimus*.

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Extruding Liquid Gobets by Asian Giant Honeybees (*Apis dorsata*) as Thermoregulation Mechanism

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The Asian Giant honeybee (*Apis dorsata*) colony constructs a large single-comb exposed nest. Layers of the worker bees aligned themselves to form 'bee curtains' to envelope the comb. It is commonly found that the outermost curtain bees regurgitate liquid gobets periodically, especially on hot and windy day. The probable role of the behaviour in nest thermoregulation is investigated.

By comparing body parts (head, thorax and abdomen) temperature of liquid gobet curtain (LGC) bees and non-liquid gobet curtain (NLGC) bees, it was found that there is a highly significant difference ($P \leq 0.01$) between the two group treatments (LGC bees - 1.72°C ; NLGC - 3.61°C , above ambient). There is a positive correlation ($r = 0.38$; $n = 10$) between the head temperature above ambient and the nectar crop volume of the LGC bees.

The liquid gobets (water/nectar) with sugar concentration ranging from 0% - 55% can be as big as the bees' head. More than half of the gobet samples (mean = 11.25 μl , $n = 10$) contained water. Investigation on the effects on regurgitation rate of the liquid gobets ($n = 53$) by directing air currents with a toy fan showed a highly significant increase ($P \leq 0.01$) from 6.37 times/min to 8.84 times/min.

The results of the experiments indicate there is a probable role of extruding liquid gobets in thermoregulation of *A. dorsata* bees.

3 DEVELOPMENT AND SOCIAL EVOLUTION

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3.1 Reproduction

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SYMPOSIUM

Towards a Unified Reproductive Biology of the *Hymenoptera*

Organizers: Alfred Buschinger and Ross H. Crozier

Introduction

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We organized this symposium because of our concern at the appearance, in recent papers dealing with apterous and more or less workerlike reproductives in ants, of contradictory definitions of "queen", "worker", etc.. These contradictions have shown that the traditional practice of defining queen and worker by both form and function may not always be satisfactory. The aim of the meeting, thus, was to bring together specialists for most of the important groups of social Hymenoptera (ants, bees and wasps), and to ask them to give reviews on certain aspects of the reproductive biology of their respective group. These aspects were the following:

1. What is the extent of the reproductive division of labor in your group?
2. When during life is the reproductive role of an individual determined, i.e., is it during the preadult or during the adult phase?
3. To what extent is reproductive role differentiation accompanied by morphological specialization?
4. Is there polymorphism within the reproductive role (e.g., mated workers versus true queens, ergatoid versus true queens, etc.)?

The lectures of the six speakers are summarized in the following pages. For primitively social bees, especially Halictidae, EICKWORT and KUKUK pointed out that a reproductive division of labor has evolved convergently several times, that it apparently has been lost again in species groups with a secondarily univoltine life cycle, that the determination of the reproductive role occurs in adulthood, and that morphologically discrete castes are rare and secondary in evolution.

In highly social bees, such as the honey bees and stingless bees, queens are physiologically and morphologically preadapted for their role as the reproductives, caste determination occurring through larval food (or a genetical mechanism in some *Melipona*), whereas in bumblebees queens are not very distinct from workers morphologically (VELTHUIS).

In the Vespidae, a caste dimorphism probably does not occur in the subfamily Stenogastrinae, whereas it is well marked in the Vespinae. In certain groups, such as *Ropalidia* (Polistinae), the extent of caste dimorphism may vary widely, reflecting diverse social organization forms (YAMANE and ITO).

For many species of bees and wasps, therefore, the specialists concerned find function to be the most approp-

riate criterion for defining castes. Otherwise, the lack of morphological differentiation between queen and worker means that no caste differences can be discerned in such species.

In ants, the situation is more complicated. For army ants (GOTWALD), the remarkable queen form, *dichthadiigyne*, is clearly definable by both form and function, as is usually the case in higher ants (BUSCHINGER). In some higher ants, however, the inseminated female reproductives exhibit a morphological range from normal gyne appearance, through intermediates, to workerlike specimens. The latter still possess fully developed ovaries and receptacles. In higher ants, therefore, the capacity for queen function is determined by the presence or absence of a spermatheca, and this is governed primarily by conditions during larval life.

Buschinger, therefore, has proposed that the caste-denoting terms queen and worker be used to define the roles, and that various other terms (*gynomorph*, *intermorph*, *ergatomorph*) denote the various morphological types.

A similar distinction between queen and worker occurs in some *Ponerinae* (PEETERS) but in other members of this subfamily all or a large proportion of the inseminated female reproductives are workerlike in morphology. The occupation of the reproductive role by these workerlike reproductives is determined in adulthood exclusively through insemination, as in some primitively eusocial bees and wasps. In some of these species, there are also morphologically distinct queens, in contrast to the bees and wasps discussed.

Peeters prefers a morphological definition of the castes queen and worker, and terms "*gamergates*" those mated and reproductive specimens which have replaced the morphological queen caste in "queenless" species.

The question of whether castes should be defined preferably by role or by morphology could not be settled to everybody's satisfaction during the final discussion of the six lectures. Thus, in order to prevent confusion or misunderstanding, each scientist involved in caste problems should carefully consider which definition she/he will follow, and explain his or her use of caste terms.

The Diversity of Reproductive Systems in Ponerine Ants

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Reproductive division of labour is a fundamental characteristic of eusocial organization. In the ants it is normally associated with the existence of a queen and a worker caste. Caste differences within a species occur when larvae follow different developmental pathways, which result in various specialized morphological traits. "Caste" is here used in its physical sense, and does not refer to roles.

Ants in the subfamily Ponerinae exhibit a variety of reproductive modifications from the ancestral pattern. It has long been known that ergatoid queens occur in many species; these are permanently wingless, and their thorax has become simplified along with the reduction of the wing muscles. As a result, ergatoid queens look very similar to workers, but their thoracic sclerites are distinct, their gaster is larger, and sometimes they retain ocelli. However the reproductive characteristics of ergatoids has remained somewhat of a mystery. Haskins and Whelden (1965) suggested that the loss of the flying ability had been paralleled by a decrease in breeding ability. Others have speculated that ergatoids are intermediate between the queen and worker castes.

Another feature of the ponerines is that, in several species, the egg-laying individuals are not externally distinguishable from the workers. Wheeler and Chapman (1922) described a worker copulating with a male in *Diacamma rugosum*, a species in which distinct queens have never been collected. They accurately pointed out that these mated workers are different from ergatoids. However, since then, the use of the term "ergatoid" has become confused, because it has been used indiscriminately in all ponerine species lacking alate queens.

The details of worker reproduction in the Ponerinae have only recently become understood, and Peeters and Crewe (1984) proposed the term "gamergate" to describe a worker which takes part in sexual reproduction. This terminology is necessary to halt the confusion with ergatoids, which are members of a queen caste despite their minor external differences. The complete loss of the queen caste is an important evolutionary event which has occurred repeatedly and independently in various ponerine tribes (Table 1).

Species with a queen caste How different are queens and workers in this phylogenetically primitive group of ants? Since queens are specialized to lay eggs, it may be useful to compare their ovaries with those of workers. In some species, queen ovaries contain more ovarioles (Table 1), while in others queen ovarioles are longer (e.g. *Onychomyrmex hedleyi*). Generally, ponerine queens have a very moderate rate of egg-laying, and this must be a major explanation for the small size of their colonies. Only in species which are polygynous is there a large nest population.

Ergatoid queens from three different tribes have been studied. In *Megaponera foetens*, there is a marked dimorphism in ovary size between queens and major workers, and this is correlated with a high egg-laying rate (32 eggs/day). In contrast, it is 4,4 in *Leptogenys nitida*.

Table 1 Characteristics of the egg-laying individuals in various ponerine species. Colony sizes are approximate. M=monogynous, P=polygynous

			colony size	ovarioles / WORKER	ovary QUEEN
TRIBE AMBLYOPONINI					
<u>Amblyopone australis</u>	alate queen	M	< 50	4	4
<u>Onychomyrmex hedleyi</u>	ergatoid	M	< 1000	3	3
TRIBE ECTATOMMINI					
<u>Rhytidoponera purpurea</u>	alate queen	P	> 1000	2	8-10
<u>R. confusa</u>	queen/gamer.	*	< 500	2-4	5-8
<u>R. sp. 12</u>	gamergate		< 500	3	-
<u>Heteroponera relictia</u>	ergatoid	M	< 100	1	4
TRIBE PLATYTHYREINI					
<u>Platythyrea schultzei</u>	gamergate		< 50	3	-
TRIBE PONERINI					
<u>Odontomachus cephalotes</u>	alate queen	P	1000	6-7	7
<u>Leptogenys mjobergi</u>	ergatoid	M	< 50	3	3
<u>L. nitida</u>	ergatoid	M	< 250	1-2	13-14
<u>L. schwabi**</u>	gamergate		< 100	3	-
<u>Paltothyreus tarsatus</u>	alate queen	?	1000	6-7	13-14
<u>Mesoponera australis</u>	alate queen	M	< 50	3	3
<u>Pachycondyla wroughtoni</u>	alate queen	M	< 500	4	4
<u>P. krugeri</u>	gamergate		< 100	4	-
<u>Megaponera foetens</u>	ergatoid	M	> 1000	12-20	30-32
<u>Ophthalmopone berthoudi</u>	gamergate		< 500	3	-

* queenright and monogynous, or with several gamergates (Ward, 1983)

**M. Zini, unpublished data

Ergatoids in L. nitida and L. mjobergi differ in ovariole number, and this is reflected in their colony sizes (Table 1). All these species with ergatoids are monogynous, indicating that mechanisms of social regulation exist. To conclude, ergatoids exhibit all the traditional attributes of the queen caste, and they must have evolved from alate queens. The adaptive reasons for this remain unclear.

Species without a queen caste In all the queenless ponerines studied, only mated workers reproduce, and these lay haploid and diploid eggs. In Rhytidoponera sp. 12, large oocytes occur in the ovaries of unmated workers, but they do not mature. In contrast, unmated workers in O. berthoudi have completely undeveloped ovaries. Gamergates have a low rate of egg production (about 1 egg/day), because they have the simple ovaries typical of the worker caste; only a few oocytes can mature simultaneously. Worker reproduction is only viable with several gamergates in each colony. The numbers of gamergates per nest vary during the year, and there is no evidence of inhibition or regulation.

Colonies with gamergates occur almost exclusively in the Ponerinae, and this is because queen and worker castes have not diverged markedly. Egg-laying workers must not be referred to as "queens".

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The Relationship of Form and Function in Army Ant Queens

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The true army ants are divided into two subfamilies, implying a convergent but separate origin for the army ant adaptive syndrome in these two groups. New World species belong to the Ecitoninae, while the Old World forms comprise the Dorylinae. Although army ants can be characterized morphologically, they are primarily defined by their behavior. Typically, army ants are group predators (i.e., they forage for and retrieve prey en masse) and they are nomadic, moving from one temporary nest site to another, sometimes with precise, predictable timing (Gotwald, 1982). Army ant queens are called dichthadiigynes or dichthadiiform ergatogynes. They are permanently wingless, have greatly reduced eyes, possess an enlarged waist and gaster, and presumably have strong legs. This queen habitus is unique among the ants (Wilson, 1971).

QUEEN MORPHOLOGY

The most conspicuous of the dichthadiigyne characteristics are the absence of wings and the enlarged gaster. Aptery in army ant queens not only influences the way colonies are founded (by swarming), but also has played a role in the zoogeography of these ants (Gotwald, 1982). The size of the queen's gaster relates directly to her ability to produce large numbers of eggs. The ovaries of at least some species are composed of large numbers of polytrophic ovarioles, and at times of egg-laying, the queen becomes physogastric, i.e., the gaster swells, exposing the intersegmental membranes (Gotwald, 1982).

Ontogeny of the queen has not been investigated. Generally, worker broods are produced to the exclusion of sexual brood. The occasional production of sexual brood forecasts that upon brood eclosion the colony will undergo fission (swarm). When sexual brood are present, a bipolar organization in the colony arises. Some workers are greatly attracted to the brood, while others remain with the functional queen. Eventually, fission is accomplished when half of colony follows the functional queen and the other half goes with one of the virgin queens. Any superfluous queens are either abandoned or killed by the workers (Schneirla, 1971).

QUEEN FUNCTION

Army ants are exclusively monogynous, the one known exception being the ecitonine Neivamyrmex carolinensis (Rettenmeyer and Watkins, 1978). The fundamental properties of insect societies probably bias species toward monogyny. Swarming, on the other hand, is most likely an advantage only if the survival of queens is enhanced when accompanied by workers (Hölldobler and Wilson, 1977). In the case of army ants, the claustral founding of new colonies by single queens in ancestral forms was lost with the increase in reproductive capacity of the queen that

evolved concomitantly with group predation. The effectiveness of group predation is positively correlated with large colony size.

The egg-laying capacity of army ant queens is extraordinary. The annual yield of eggs from a single queen in the doryline *Dorylus wilverthi* was estimated to be 36 to 48 million (Raignier and van Boven, 1955). Unless sexual brood are already present, death of the queen will bring about the demise of the colony. Although workers possess ovaries, they apparently produce only trophic eggs (Gotwald, 1982).

Presumably, all army ants are nomadic, although the frequency with which nest sites are abandoned is unknown for most species. A minority of species have a distinct functional cycle of alternating statary and nomadic phases. Brood and callow worker stimulative cues appear to activate and sustain nomadic behavior (Schneirla, 1971).

When emigration occurs, the queen is no longer physogastric. This assures that the intersegmental membranes are not unduly exposed to possible abrasion and puncture. The queen moves under her own power and is accompanied by a retinue of workers (Rettenmeyer et al., 1978).

CONCLUSIONS

The dichthadiigyne is uniquely adapted to army ant lifeways:

1. Egg-laying capacity, as evidence in ovarian development, is well adapted to the production of large numbers of workers and thus to the maintenance of large colony size. Large colony size, in turn, is prerequisite to effective group predation.
2. Because the queen can produce large quantities of eggs in a short period of time, she is adapted to a nomadic existence.
3. The fact that the queen has well-developed legs and that she is not physogastric at time of emigration means that she can move under her own power to a new nesting site.
4. The development of dichthadiigyne morphology has compromised the ability of queens to act as single, claustral foundresses, and led to the evolution of swarming in the army ants.

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Polymorphism and Reproductive Division of Labor in Advanced Ants

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Clearly defined female castes are common in the higher ants. The queens usually are larger than the ♀♀, have deciduous wings, and a generally hymenopteran thoracic skeleton. The ♀♀ are (much) smaller, have a narrow thorax, and the thoracic sclerites are widely fused. Size differences vary between moderate and enormous. Many species have a worker polymorphism, and particular subcastes, e.g., soldiers may be present. Caste polymorphism in the vast majority of ants is concurrent with reproductive division of labor, in that queens are the only specimens having large ovaries with a spermatheca. ♀♀ often also have ovaries, and may lay eggs which develop into ♂♂. A receptacle is lacking in most ♀♀. Caste determination is achieved usually in the preadult instars, through food and other environmental influences (Wilson, 1971). Among the higher ants, however, a growing number of instances come to light which remarkably differ from the usual conditions:

(1) Thus, in Myrmica and Leptothorax species quite frequently dealate ♀♀ occur in colonies, who are not inseminated or reproductive (Buschinger, 1968). Strictly speaking, for such ♀♀ the ultimate decision on their reproductive or worker role occurs in the adult instar.

(2) In the myrmicine genus Formicoxenus ♀♀ occur in an intergraded series of workerlike to fully alate specimens. The alates and all intermediates down to apparent workers have well-developed ovaries and receptacles. All these forms may be inseminated and may become the reproductives of colonies. The Formicoxenus species also exhibit a functional monogyny. Thus, colonies exist with an essentially workerlike reproductive, and some intermorphic or even dealate ♀♀ working for her (Francoeur et al., 1985).

(3) In the slavemaking ant, Harpagoxenus sublaevis, the reproductive caste is dimorphic, with a rare alate form and a much more common workerlike or slightly intermorphic one. The alate ♀♀ are homozygous for an allele "e", whereas the "ergatoid ♀♀" are homozygous for "E", or heterozygous. Larvae of all three genotypes may develop into ♀♀ without a spermatheca, or into potential reproductives, due to usual determining factors like food. The two alleles, however, have effects also on caste or role determination, in that "E" brings about a strong bias towards worker development, whereas ee- or eE-larvae much more frequently develop into alate resp. workerlike reproductives (Winter & Buschinger, 1986).

These three examples, as comparable cases among the Ponerinae, raise questions on behalf of traditional caste

terminology. Following Wilson (1971) caste is "any set of individuals...that are both morphologically distinct and specialized in behavior". Obviously this definition is not satisfactory in the examples mentioned. Michener (1974) provides another definition: "Castes in Hymenoptera...are the forms of females that perform different functions in a colony. They differ...at least behaviorally and physiologically, often also in structure". This definition, with emphasis on role, is appropriate not only for bees and wasps, but also for the unusual cases among ants.

In addition, the essential characteristic of social insects is their reproductive division of labor, hence a role difference. We have proposed, therefore, to restrict the caste-denoting terms queen and worker to their functional meaning (Buschinger & Winter, 1976), and for the different forms of ♀♀ we have coined the terms "gynomorph", "ergatomorph", and "intermorph". The traditional use of "queen" and "worker" in ordinary social Hymenoptera will not much be affected, the new precise terms being necessary only when, e.g., gynomorphic workers or ergatomorphic queens are found in a given species.

The new terminology, and the restricted definition of caste = role, are applicable to all social Hymenoptera; they should contribute to a better mutual understanding of those who are working in different hymenopteran groups, and they can help to avoid confusion, e.g., in the discussion of polygyny, or "queenlessness" (Crozier & Pamilo, 1986, Itow & al., 1984).

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Alternative Reproductive Strategies in Workers of the Slavemaking Ant *Harpagoxenus sublaevis*

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Workers of the monogynous slave-making ant *Harpagoxenus sublaevis* can increase their inclusive fitness by procuring slaves to rear their kin or by producing males. This study analyzes the tension between these two reproductive strategies by assessing the impact of worker reproduction on the social structure of colonies, the division of labour, and sex investment ratios.

METHODS

The study population is situated in coastal pinewoods near Bröms, Sweden. Behaviour within colonies and on slave raids was studied by observing and filming marked individuals. Colony censuses and ovarian dissections were performed to measure sex ratios and the frequency of fertile workers. Intra-colony relatedness and the proportion of worker-produced males were estimated by electrophoretic allozyme analysis, in collaboration with Dr. T. van der Have, University of Utrecht.

RESULTS AND DISCUSSION

In *H. sublaevis* colonies the queen and a small physically dominant elite of workers form a stable, linear dominance order in which rank is correlated with ovarian development and the frequency of trophallaxis with *Leptothorax* slave workers. This discovery suggests that it pays potentially fertile *H. sublaevis* workers to intimidate their fellows (to inhibit rival reproductive activity) and to seek extra food for egg development. Allozyme analysis shows all workers within a colony are full sisters.

Ovary-developed workers spend significantly less time scouting in search of slaves, and participate less in slave raids, compared to workers without ovarian development. They therefore protect their reproductive futures by avoiding risks outside the nest.

Most worker reproduction appears to occur in orphaned colonies, which have the highest proportion of fertile workers and constitute 21-30% of all colonies. Orphaned workers produce c. 22% of all males.

In 1985 the mean proportionate dry weight investment in queens was 0.61, which was not significantly different from 1:1 investment. The observed female bias conceivably resulted from sex ratio compensation by queens in response to worker male-production, or partial worker control over sex ratio (hitherto ruled out in slave-makers), or both.

In *H. sublaevis* individual-level selection on workers to produce males is consequently a powerful influence on the colony's social structure, nutrient flow, and division of labour, even though the level of intra-colony relatedness (and hence the predicted degree of social co-operation) is maximal. Worker reproduction is clearly favoured by colony orphanage. Worker reproduction could be of general importance in ants and other social Hymenoptera.

Colony Founding and Role of Parthenogenesis in *Cataglyphis cursor* Ants (Hymenoptera - Formicidae)

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Cataglyphis cursor is a monogynous and monodomous species (Cagniant 1976a, Lenoir and Cagniant 1986). In the Mediterranean region of France, swarming takes place in late June - mid-July. Alate females run near the nest entrance of their colony while males fly off their nest. The copulation occurs after a nuptial course (Cagniant 1976b). Later females walk into their nest where they lose their wings, they can leave the nest again the following days. Generally they are inseminated, but it is not always the case. As a consequence, during this post-swarming period which lasts a few weeks, polygynous nests can be found in the field.

In the laboratory, experimental foundations with newly inseminated queens are always unsuccessful. If a few workers are added to the queen the result is identical. As isolated females were never found in the field independent foundation does not seem to be possible with this species.

Consequently we considered the existence of fission to explain the propagation of the colonies. Examples of fission were observed in the field : workers transport other workers and brood to an empty hole which is turned into a new nest. The transport lasts generally a few days with a permanent traffic between the two nests, and up to 3 nests can be formed from a mother nest. A newly inseminated queen is then transported to the new nest. Some of these new nests are abandoned before the transport of a queen. The mother nest can also be abandoned. Finally we have close kin societies and for several weeks workers continue to pass from one nest to the other. It is a momentaneous polycaly, which can be observed in August. If no form of fission occurs the supernumerary queens are rejected by the workers. The polygynous colonies have been observed in the laboratory where they can carry on until wintering after which monogyny is always re-established. Fission is exceptional in monogynous species which found generally new colonies with isolated females.

Cagniant (1973) discovered that *C. cursor* can reproduce by thelytokous parthenogenesis in the laboratory when colonies are orphaned. As orphan colonies were never observed in the field Lenoir and Cagniant (1986) speculated that groups of workers formed by fission could produce females this way. The observations of the transport of queens related here lead to reject this hypothesis or at least to consider that as a mere accessory mechanism. To test the role of parthenogenesis 22 colonies were orphaned in the field artificially in early May before the first egg-laying of the females. In July 7 of them were found exactly at the same spot with a functional queen. We can suppose that these queens were produced by parthenogenesis but this needs to be confirmed. This result may indicate that thelytoky could be efficient in the field but parthenogenesis seems to be only a substitution mechanism in the case of the death of a queen.

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Reproductive Castes in Primitively Eusocial Halictid Bees

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Primitively eusocial behavior has evolved in 9 lineages of the Halictinae (Halictus, Seladonia, Evyllaesus, Dialictus, Augochlora, Augochlorella, Pereirapis, and one species each of Lasioglossum s.s. and an African subgenus of Lasioglossum s.l.). All but two of these lineages also contain solitary species, so reproductive castes have evolved independently and/or been lost multiple times in the sweat bees.

Two basic patterns of social organization are apparent in the Halictinae (Michener, 1974). In the more "advanced" pattern exemplified by Evyllaesus malachurus, workers and reproductives are produced in discrete broods, castes are typically distinct, and queens live through the life of the colony. In the more "primitive" pattern exemplified by Dialictus zephyrus and D. lineatulus (Eickwort, 1986), there is no separation between the production of worker and reproductive broods, queen and worker castes overlap broadly in size and physiology, and queens frequently die before the production of new reproductives in the nest and are replaced by sisters of the workers.

Studies in observation nests of D. zephyrus indicate that castes are determined by behavioral interactions among adult bees. Queens are the primary reproductives in a colony, but they do not completely suppress ovarian development or mating by workers. The first female to become established in a nest is most likely to become the queen; size only becomes a factor in caste determination among equal-aged bees. This is supported by field observations of both advanced and primitive societies: Daughter queens that replace foundresses are not significantly larger than their worker nestmates. Queens are more clearly defined by behavior (especially dominance interactions and absence of foraging) than by characters obtained through measurement and dissection.

Field studies of diverse halictines indicate that there is no close correlation between caste size differences and tendency of workers to mate or lay eggs. Moreover, significant differences in these measures of social structure exist among populations of the same species that occur in different ecological settings (e.g., in Halictus farinosus, Eickwort, 1985), as well as during the season within single populations. Species should not be placed on an evolutionary spectrum based on social organization.

Facultative pleometrotic associations of nest foundresses are common in sweat bees. In D. lineatulus, all foundresses are inseminated and exhibit ovarian development, but the largest bee typically has the best developed ovaries and does not forage. In other species, the foraging cofoundresses often exhibit greatly reduced ovarian development.

Semisocial associations are thus common both in first-brood pleometrotic nests and in second-brood nests headed by replacement queens. Semisocial species of Augochloropsis and Pseudaugochloropsis that do not develop eusocial colonies appear similar to these in social structure.

Not all halictine species exhibit castes that readily fit the above models. In tropical and subtropical Halictus ligatus and in California H. farinosus, over half of the workers exhibit ovarian development and/or insemination, and foragers are frequently queen-like reproductives (Packer and Knerer, 1986, Eickwort, 1985). Conversely, tropical and desert Seladonia may have huge nests with many hundred bees (589 in S. lutescens), but castes are distinct, with a few very large foundress queens or small replacement queens (e.g., Brooks and Roubik, 1983).

CASTE TERMINOLOGY

GYNE: potential nest foundress

FOUNDRESS: initiator of a nest

Cofoundress: one of two or more females that initiate the same nest

Dominant Cofoundress: reproductive cofoundress that does not forage

Subordinate Cofoundress: foraging cofoundress in nest with dominant cofoundress, may or may not be reproductive

QUEEN: dominant, reproductive female in a second-brood or later nest

Foundress Queen: queen that was present in the first brood period

Replacement Queen: queen that is a daughter of a foundress

Unmated Queen: uninseminated replacement queen

WORKER: subordinate female in a second-brood or later nest; ovarian development and mating present or absent

Sterile Worker: uninseminated, ovaries undeveloped or lumpy

Laying Worker: uninseminated, ovaries with oocyte over half mature

Mated Sterile Worker: inseminated, ovaries undeveloped

Mated Laying Worker: inseminated, ovaries with developed oocyte

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Caste Differentiation and Egg Laying in the Highly Social Bees

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The central topic of this symposium is scientific terminology. The distinction I would prefer to make between primitively social and highly social (i.e. species having real queens and workers) colonies is whether during evolution a different ontogenetic program for producing queens and workers was developed. This leads to an irreversible bimodality in the properties of individuals within the colony. In primitive social insects the differentiation between individuals is flexible and is based on continuous variation in properties within the population.

The bimodality is not necessarily expressed in morphology. Probably, in evolution, physiological differences with respect to the ability to mate or to hibernate preceded the morphological expression of the differentiation process. It is always environmental cues that provide the information which triggers the use of the alternative developmental pathways (that are probably genetically programmed).

Highly social bees

To avoid discussions which bees are highly, and which are primitively social I will restrict myself to the bumblebees, the true honeybees and the stingless bees. These all have monogynous colonies, except for a few *Meliponids* that can have more than one queen. In some bumblebees the queen and the worker differ only in size, in other species their morphology is qualitatively different.

Caste differentiation

In the highly social bees the food quality or the quantity available to a larva provides the cue for caste differentiation.

In the mass provisioning stingless bees no qualitative factors became known so far. Determination, therefore, is exclusively based on the amount of food and will take place only at the end of the feeding period. In the genus *Melipona* queens emerge from the same kind of cells as do workers and differences in food quantity are only small. A very sensitive mechanism apparently regulates the differentiation process. In addition only 25% of the larvae respond to this food quantity. Kerr suggested these larvae to be heterozygotic for two queen-determining genes.

In the other stingless bees the difference in food quantity for worker or queen is more pronounced. In *Scaptotrigona* special queen cells are constructed which contain 2-3 times as much food as found in a worker cell. In *Leurotrigona* a larva may obtain additional food from a neighbouring young cell by piercing the wall, thereby doubling its food intake, which leads to queen development.

Bumblebees and true honeybees are progressive provisioners. In bumblebees the amount of food ingested during the final larval instar

is decisive for queen differentiation. The frequencies by which workers feed a larva in its last instar is quite different for developing workers or queens. Some bumblebees, for instance *B. terrestris*, have an additional mechanism in that the queen suppresses queen differentiation in her female offspring as long as she is dominant.

In the honeybee the combination of special cells and progressive provisioning enables an early differentiation. Larvae in queen cells are fed royal jelly, a secretion that worker larvae obtain in limited amounts and then only for the first 3 days of larval life. Plasticity in the developmental program is restricted to these 3 days.

In all cases the queen and worker caste is monomorphic. In some stingless bee species the unique feature occurs of males coming in two sizes: normal and giants, but their specific functions are unknown.

Worker egg laying

In all three groups of bees workers produce eggs, but major differences exist with regard to why and when. Workers, being unmated, produce haploid eggs. A first evolutionary stage, therefore, is their contribution to the production of males. This implies competition with the queen and among workers.

Queens of bumblebees and honeybees reduce the rate of oogenesis in their workers and also inhibit actual egg laying for a long time once eggs are formed. In bumblebees, where this is less complete, workers will start laying viable eggs and will produce sons. This is the last phase of the colony cycle, characterized by the loss of queen dominance, overt aggression and mutual egg eating. In honeybee workers egg laying occurs only when the queen has been away for some weeks. Here too most eggs are wasted. Inefficiency is among the most distinct aspects of the production process.

Stingless bees are rather variable with respect to worker egg laying. *Frieseomelitta* workers never activate the ovaries, *Leurotrigona* workers do so only under queenless conditions. In most species, however, workers produce eggs without any queen inhibition. Queens control worker reproduction by interfering in the process of laying. They often eat worker eggs. This stands at the basis of the marvellous evolution within the stingless bees of trophic eggs, which even may be without a nucleus, and the associated behaviour of laying them. At the same time such a species may have fertile worker eggs. The success of workers depends on the timing: is the egg ready to be laid, is it laid at the proper moment in colony-dependent processes and in the right place? In some species most males stem from workers, in other species this depends on the season or colony conditions.

Apart from such processes in queenright colonies, these species have also laying workers if queenless. Now the behaviour is often distinct, being more overtly aggressive and competitive.

The assumption that laying workers contribute to the gene pool, is superficial. If the reproductive system of a species includes worker participation one might expect efficiency. Efficiency in the rearing but also in the timing of male production related to season. In outbreeding species colonies should mutually synchronize the emission of sexuals, probably by responding to environmental factors. In many bees, however, worker derived males are out of season. Evolution went beyond the point where males are real reproductives. Their occurrence reveals the mechanism of colony organization still to be based on reproductive competition, but their reproductive potential approaches nil.

Caste Differentiation and Social Organization in the Old World *Vespidae*

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The queen/worker dimorphism is compared among 3 subfamilies (Stenogastrinae, Polistinae and Vespinae) of the Old World Vespidae in relation to social organization, based on known morphometric and biological information.

Caste dimorphism: This probably does not occur in subfamily Stenogastrinae, the most primitively social group among the Vespidae, while it is greatest in Vespinae having highly organized societies (Table 1). Subfamily Polistinae show the greatest diversity in the Q/W dimorphism. Genus *Ropalidia* is particularly interesting because it involves varying degrees of caste differentiation. Observed species of subgenus *Icariola* show no or weak dimorphism as in *Polistes*. On the other hand, subgenus *Icarielia* developed a distinct dimorphism, being greatest among the Old World Polistinae and comparable to that of *Stelopolybia* of the New World, though still less than in the Vespinae (Table 1). This strongly suggests that *Icarielia* has established highly organized societies.

Colony founding: There are two types of colony founding in the Vespidae. The independent-founding, in which single or multiple foundresses initiate colonies, is seen in the Stenogastrinae, most Vespinae and most Polistinae (*Polistes*, *Parapolybia*, *Belonogaster* and some subgenera of *Ropalidia*) of the Old World. The swarm-founding, in which a proportion of the queens and workers migrate elsewhere to found a new colony, is believed to occur in some subgenera of *Ropalidia* (*Icarielia*, *Ropalidia*, *Anthreneida*, etc.) and *Provespa* (Matsuura & Yamane, Sk. 1984), though we have no direct evidence.

Monogyny/polygyny: Among the Old World species, only the swarm-founding type *Ropalidia* seem to have multiple queens throughout the colony cycle (= permanent polygyny). Other groups are monogynous (Vespinae, *Polistes*, *Parapolybia* in temperate zones, some stenogastrines) or temporarily polygynous (*Polistes*, *Parapolybia*, *Belonogaster*, many

Table 1. Comparison of Q/W dimorphism in *Ropalidia* and other groups.

Species	Q/W ratio of alitrunk length	No. Q/Q+W (%)
<i>Icariola marginata</i> ¹⁾	No distinction	1 to several (in large colonies) functioning Q/colony
<i>Io. variegata</i>	No distinction	1 to several (in large colonies)
<i>Anthreneida sumatrae</i>	1.06	2.8 %
<i>Icarielia montana</i> ²⁾	1.14	0.21, 0.58
<i>Ie. flavopicta</i>	1.17	4.2, 22.3
<i>Ie. romandi</i>	1.04	0.2
<i>Stelopolybia areata</i> ³⁾	1.15	5.5, 12.2
<i>Vespula germanica</i> ⁴⁾	1.37	Single

1) Gadgil & Mahabal (1974); 2) Yamane et al. (1983); 3) Jeanne & Fagen (1974); 4) Blackith (1958: thoracic width).

Ropalidia in the tropics, some stenogastrines). In many cases of multifemale-founding, however, colonies have single functional queens (= functional monogyny). In some Icariola species, large mature colonies have multiple egg-layers (Yamane 1986), probably caused by insufficient control by the queen over ovipositions by her nest mates.

Colony cycle: Temperate and subtropic species have annual and synchronous colony cycles, while many tropical species, e.g., Parischnogaster mellyi and R. (Icariola) variegata, have asynchronous cycles lasting more than 1 year by means of queen supersedure (Yamane 1986).

Evolution of different life styles in Ropalidia and Vespinae: The combined system of swarm-founding and permanent polygyny is regarded as one of the most advanced stages of social evolution in social wasps. It has probably evolved independently in the Old and New Worlds.

In R. (Icariola) variegata the founding groups sometimes involve uniseminated, worker-like females, and we regard this as a very primitive state of swarming. In a more advanced phase, the size of founding groups and the ratio of attending workers might increase. Icarielia species, which show a distinct Q/W dimorphism, are placed on the most advanced phase among the Old World Polistinae. The swarm-founding seems to be a response to high predation pressure during the pre-emergence period in the tropics. It also enables long-distance migration of new colonies, which decreases intercolony competitions (West-Eberhard 1982). The occurrence of multiple queens gives a higher reproductive capacity per colony and realizes huge colonies. Defence by a large colony population is apparently effective against attacks by big visually-hunting animals. Further, in the tropics where the colonies are often disturbed and forced to abscond elsewhere, possession of multiple queens, each with a low reproductive capacity but high movability, may be more adaptive than with a single gravid queen.

The vespines, which extended their distribution to cooler zones, have also realized the colony gigantism, however, they adopted a different means. Their colonies, as a rule, depend on single queens who have larger numbers of ovarioles, each of which can produce far more eggs than the Polistinae.

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Division of Labour and Nestmate Interactions in Two Species of Allodapine Bees

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In some African allodapine bees, a primitively eusocial stage occurs during the annual life cycle: singly-founded spring nests contain several females plus brood by mid-summer. In this study, direct observations were made on naturally established eusocial colonies of *Allodape exoloma* and *Braunsapis foveata*, species with similar life cycles. Division of labour in foraging and guarding, and food related interactions of nestmates are reported here.

METHODS

Nestmates from mid-summer colonies were marked and replaced into glass observation nests with three to six females in each. After 2-5 weeks of observations, females were dissected and identified by reproductive condition and relative wing wear as queen or workers 1-5. Nestmate participation in four behaviours are compared for 9 *A. exoloma* and 7 *B. foveata* nests.

RESULTS

In both species, strict division of labour separated nestmates into foragers and non-foragers. In *A. exoloma* the caste of the primary forager varied, but was usually the queen or the oldest worker. In all seven *B. foveata* nests, the oldest worker was the primary forager. In contrast, a more moderate division of labour was characteristic of guarding as well as other intra-nidal tasks, so that in most nests of both species, all nestmates participated.

In trophallaxis, almost all (93%) of the bees donated as well as received nectar. Although primary foragers were the most frequent donors in trophallactic exchanges, non-foragers were donors in 25% of 190 *A. exoloma* and 34% of 359 *B. foveata* exchanges.

In colonies successfully rearing larvae (*A. exoloma* n=2, *B. foveata* n=5), all nestmates participated in brood care. Two aspects of brood care were feeding larvae and "tonguing" larvae (i.e. drinking nectar stored on a larva's venter). Primary foragers fed larvae more than tongued them, but other nestmates tongued more than fed the larvae.

In conclusion, the two species were similar in division of labour: foraging was restricted to 1 or 2 adults per nest, but all nestmates acted as guards. The comparatively greater activity of *A. exoloma* queens in both foraging and guarding suggests that division of labour is not as well established in this species as in *B. foveata*. Food related interactions in both species showed that nestmates shared nectar through storage of nectar on larvae as well as through trophallaxis.

The Tropic Eggs of Colony Founding Ant Queens

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The laying of trophic eggs by workers, in queen-right societies, has been reported in many ant genera of both primitive and advanced subfamilies except Pseudomyrmecinae, Leptanillinae and Dorylinae. The laying of trophic eggs by colony-founding queens is known only in 3 genera (*Atta*, *Solenopsis* and *Tetramorium*) and that by virgin queens in 2 genera (*Solenopsis* and *Pheidole*) all of which are within the subfamily Myrmicinae. In this paper a similar phenomenon is recorded for the first time in claustrally colony-founding Formicininid queens; in 5 species of *Lasius* (*niger*, *hayashi*, *sakagamii*, *productus* and *sonobei*) and in *Paratrechina flavipes*.

Queens were collected immediately following the nuptial flight or copulation and were individually maintained at 25°C in plastic Petri-dishes with a piece of water-soaked cotton. The numbers of each brood were counted every day. Live, dry and fat-free dry weights of queens and broods were determined from randomly selected sample specimens.

Queens initially lay a batch of 20-40 reproductive eggs and then switch to laying trophic ones. These 2 types of eggs are piled in one cluster and, after the hatching of larvae, the trophic eggs are rapidly consumed by larvae of all 4 stages. The respective differences between the reproductive and trophic eggs; fertile and infertile, bean-shaped and ellipsoidal, rigid and soft chorionated, high water content and low, etc., are similar to those seen in the workers of some species (e.g. *Myrmica*, BRIAN & RIGBY 1978 Ins. Soc., 25:89-110). Experimental evidence showed that the queen can discriminate between these two egg types. Removal of only reproductive eggs resulted in replacement via oviposition of a similar number of reproductive eggs, whereas, continuous removal of trophic eggs resulted in only a delay in larval growth, probably due to the consumption of newly laid trophic eggs by investigated larvae immediately prior to their removal. Ecological energetic calculations employing the reported respiration rate of ant larvae (1.13 $\mu\text{l O}_2/\text{mg dry weight hr.}$ NIELSEN 1986, J. Ins. Physiol., 32:125-131.) showed that the trophic eggs represented an adequate nutritional source for the growth and respiration of larvae.

The absolute dependence of larvae upon the trophic eggs for nutriment was not proved by observation on the behaviour of the queen and larvae, but whatever the degree of dependence is, the regulated laying of trophic eggs by the colony-founding queen is an important evolutionary trait in the reproduction of ants.

3.2 Caste Differentiation

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Endocrine Control of Caste Determination in Some Social *Hymenoptera*

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One of the most striking features of insect societies consists in the morphological and/or behavioral differentiation of individuals within the same sex into castes.

In social Hymenoptera, the phenomenon is restricted to the female sex. What conditions are responsible for this differentiation? Extensive investigations have been devoted to finding an answer to this question. One of the main fields is the study of internal factors, especially, the study of the influence of morphogenetic hormones (i.e. Ecdysteroids and Juvenile hormones, JH).

Modulations of these hormones occurring at certain critical periods during development cause differentiation into either one caste or the other (Nijhout and Wheeler 1982). Most of the studies have been based on injections of JH or JH analogues as well as topical applications that permit the definition of sensitive periods during which variations in the hormonal balance lead the insect to switch to a defined caste. Before that period, the insect is bipotent. A further stage in such investigations consists in measuring the levels of circulating hormones in order to test hypotheses suggested by previous experiments.

The present paper summarizes some measurements made on a few species of bees, ants and wasps which provide a first sight on the problem.

RESULTS

I BUMBLE BEES. In these primitively social bees, two kinds of caste determination were found to occur (Röseler, 1976, 1977). In *Bombus hypnorum*, worker larvae and even young prepupae up to the age of 24 h can be induced by JH application to develop into queens. In *B. terrestris*, the larvae are determined as soon as the first instar to develop either into a queen or a worker. The differentiation of workers cannot be changed but the presence of a dominant queen in the colony redirects the development of a queen larva to a worker, even during the last larval instar. JH was found to be ineffective in caste determination of this latter species.

We chose to study the titers of both JH and ecdysteroids during prepupal development which period was shown to be sensitive in *B. hypnorum*. In *B. terrestris*, this sensitive period follows the time when a dominant queen can inhibit larval determination to a queen.

The results (Strambi et al., 1984) showed that in both species a peak of ecdysteroids and a peak of JH occurred almost simultaneously. Workers had a shorter prepupal development than queens with the hormonal peaks occurring at about the middle of this stage. In queen prepupae, the hormone levels peaked during the third quarter of the stage. Thus we could determine a "worker pattern" and a "queen pattern". As we always measured ecdysteroids and JH from the same

sample of haemolymph extracted from a single prepupa, it was easy to compare the precise timing of the two peaks. In the two castes of both species, the JH level increased more than the ecdysteroid level at the beginning of the stage but ecdysteroids peaked a few hours earlier.

In B. terrestris (which seems not to be JH sensitive) the JH levels were very close in worker and queen prepupae. As for B. hypnorum, the individual JH levels showed greater variation than in B. terrestris and the peaks were smaller in workers than in queens. This difference might reflect a causal relationship between JH and the differentiation into queens which occurs in that period.

It will be of interest to extend these investigations to earlier larval stages and to the pupal stage to determine whether the caste specific developmental program reflected by the hormonal patterns is present during other stages of development.

II ANTS. We applied the same method to two ant species, i.e., after defining a sensitive stage, we determined hormonal levels. We studied two different species chosen for their rather different biology Plagiolepis pygmaea and Pheidole pallidula (Suzzoni et al., 1980, 1983). However, the small size of the animals did not allow us to work on haemolymph, so we assayed only ecdysteroids from whole body extracts.

P. pygmaea. In this species, post hibernation third instar larvae are bipotent; in queenless colonies they develop into queens, but in the presence of a queen, these larvae become workers (Suzzoni, 1983). Therefore we chose to study the time course of ecdysteroid titers in third instar larvae developing into workers or queens.

In queen-biased larvae, the ecdysteroid levels showed only slight variations. The highest values were recorded when the larva reached about one-third of its final weight. In worker-biased larvae, the ecdysteroid levels grew to about 8 times the level observed in queen-biased larvae. The maximum values were recorded when the larvae reached about half their final weight. As we do not know the pattern through time of the growth in weight, it is impossible to say whether the peak in workers occurs earlier or later than in queen larvae. In both castes, the levels fall to low values at the time of cocoon spinning. Here, we recorded higher values of ecdysteroids in the worker caste.

Pheidole pallidula. In this species, the critical period occurs much earlier than in P. pygmaea. The first eggs laid by an overwintered queen hatch into either queen or worker larvae (Passera, 1979). By the end of the first month after hibernation, all the eggs laid give workers. The period of mixed laying can be extended by topical application of JH both to newly laid eggs and to the queen herself. This means that the egg is sensitive to the action of JH throughout embryogenesis and is even sensitive via the ovaries of the queen.

In this species, we studied the ecdysteroid contents of queens laying 69 to 100 % queen-biased eggs just after overwintering and also of queens laying 100% worker-biased eggs. We found the content in ecdysteroids to be much higher in queens laying worker-biased eggs than in queens laying queen-biased eggs. As we assayed ecdysteroids from whole-body extracts (including ovaries) we are unable to say which organ (ovaries, haemolymph, fat body) the ecdysteroids came from, but the highest level in queens laying worker-biased eggs was also found if we examined the products comigrating with ecdysone and 20-hydroxyecdysone after a TLC separation.

The sensitive period seems to occur during embryonic development. Therefore, we studied the ecdysteroid content of batches of 20 to 50 eggs throughout the 7 days of embryonic development. At all times, the ecdysteroid levels were the lowest in queen-biased eggs, somewhat higher in worker-biased eggs laid just after overwintering and the highest in worker-biased eggs laid two months after hibernation. Thus, here again, development into workers goes along with high ecdysteroid content.

In the genus Pheidole a second sensitive period occurs during larval development. Within the worker caste a dimorphism exists leading to minor workers and larger big-headed workers called soldiers. In P. carinata, Wheeler and Nijhout (1981) could alter the distribution of adult sizes by the application of JH to larvae.

From a comparison of these two species, we may conclude that ants appear to have developed different strategies for queen/worker differentiation. In Plagiolepis, the differentiation occurs in the last larval instar, as in most primitive bees such as Bombus hypnorum. In Pheidole on the other hand, the differentiation occurs as early as during embryogenesis. In this case, the embryonic stage is sensitive to an hormonal influence and later on, the larval stage will be sensitive to the same hormones for a worker/soldier differentiation.

Plagiolepis workers have only small size variability (monomorphism) whereas Pheidole workers are strongly dimorphic. Further studies on the endocrinology of species having polymorphic workers should provide interesting data for better knowledge of the existence and timing of sensitive periods among the various ant species.

III WASPS. In the subfamily Vespinae, caste determination occurs during the larval stage, but the endocrinology of this has not been studied as far as we know. It results in the production of large queens and smaller workers as in the Honey bee.

In more primitive subfamilies like the Politinae a difference between queens and workers hardly exists, as the sizes of individuals that live only until winter (workers) and individuals that overwinter (foundresses) largely overlap. But some species of Polistes develop a special characteristic which consists of subcastes within the foundress caste. This subcaste determination occurs during the adult stage. In polygynic nest foundations, a linear hierarchy among foundresses is established. The dominant female will become the only egg-layer of the colony while the subordinate(s) will assume most of the social work. Several experiments (Röseler et al., 1980, 1984, 1985, Röseler, 1985) have demonstrated that the social hierarchy is established after overwintering under the influence of hormones and especially JH as estimated through its biosynthesis in vitro and through measurements of the volume of corpora allata. Moreover, injections of JH and of 20-hydroxyecdysone significantly increased the chances of the treated female to attain a dominant rank. In turn, within a few days, the social life inhibits the hormonal activity of the subordinates. These mechanisms ensure the founding society an efficient reproductive capacity.

Through these few examples, we have shown that the Insect morphogenetic hormones (JH and ecdysteroids) are involved in caste determination at various developmental periods according to the species. Both hormones show caste related variations which can be evidenced in larval instars or even in embryos. Beside these morphogenetic influences, the same hormones can alter, in primitive

social wasps, the reproductive capacities of females through an action on social behaviour. All these actions modify both behaviour and reproduction which are the main characteristics of castes in social Hymenoptera.

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Reproduction and Caste Development in Social Bees

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SOCIOEVOLUTION OF QUEEN AND WORKER FUNCTIONS IN REPRODUCTION

In the social bees many of the caste-specific tasks and functions have to be interpreted as being a consequence of reproductive specialisation by the females. One or a few of them become queens and remain fertile, and thereby often attain a remarkable fecundity. Some or many others become workers and are less fertile or even unfertile. Besides these differences in the reproductive capacity of the adults - which on the highly eusocial level are associated with morphological, physiological and behavioral peculiarities - caste formation during development became regulated in the course of socioevolution. From the multiplicity of questions involved our studies are principally concerned with problems of physiology. The main subjects used by our group are honey bees, stingless bees and more recently also sweat bees. The physiological basis for egg production - occurrence, rate of synthesis and incorporation of yolk proteins or vitellogenins - are parameters to quantify female bee fertility. I will discuss in this review some of our respective results from the viewpoint of a developmental biologist. Methods will not be explained in detail.

Within the today existing species of bees, all evolutionary steps from solitary life up to highly eusocial and populous colonies consisting of many thousand individuals are represented. It is interesting to note that even in a number of basically solitary species sometimes two females - unrelated or mother and daughter - may share a nest. The immediate consequence is the partial or complete suppression of reproduction in one female by the other. Such a case has recently been described in Japan for a *Ceratina* (Sakagami and Maeta, 1984). Advanced levels of reproductive caste differences are found in Halictine bees. Queen dominance plays an important role here (Smith and Weller, 1987). Unfortunately the physiology of the different females in such mini-colonies has until now not been studied.

REGULATION OF VITELLOGENIN SYNTHESIS IN QUEENS

Queens in honey bees and stingless bees are egg-laying machines. The rapid and permanent vitellogenin uptake by micropinocytosis into growing oocytes can be best demonstrated by autoradiography of ovary sections. Using labelled amino acids as precursors, we obtained a picture of vitellogenin coming from the fat body and being transported by the hemolymph to the ovarioles where it is finally incorporated into the ooplasmic yolk spheres of vitellogenic follicles. An oocyte is filled up with yolk material in the form of many vitellin-containing vesicles within 2 or 3 days (Engels, 1973).

If we separate hemolymph proteins using the various electrophoretic techniques, the honey bee vitellogenin can be shown to be a high molecular weight (180 kd) lipo-protein with some carbohydrate moiety. Comparing the vitellin in eggs and ovaries with the hemolymph vitellogenin, only some minor differences in charge were detected.

In adult queens the rate of vitellogenin synthesis is correlated to

actual egg production which may range from a few eggs per day up to a few thousand. But surprisingly in a honey bee queen - in contrast to other insects - vitellogenin synthesis never drops to a zero level, even during winter when no eggs are laid (Engels, 1974). A queen is always ready to produce bees. In queens laying many eggs, the synthesis of vitellogenin may reach 80 - 90% of the total hemolymph protein synthesis. So there is a pronounced quantitative regulation. The question of the factors controlling this regulation arose. It was first thought that - as is the case for many insects and some Hymenoptera, e.g. the bumble bees (Roeseler and Honk, 1987) - in the honey bee juvenile hormone (JH) could also control female fertility. Some observations on vitellogenin occurrence in gynandromorphic bees (Engels et al., 1975) seemed to support this hypothesis. But allatectomy - removal of the only known source of JH - did not suppress vitellogenin synthesis, reduce the vitellogenin titer much or terminate egg production. Even if young virgins were allatectomized soon after emergence, they were later capable of producing eggs, although some behavioral aberrations were observed. Such queens sometimes started egg laying before the mating flight which is quite unusual in bees. Also the so-called chemical allatectomy by application of precocene, an inhibitor of corpora allata functions, only affected the vitellogenin metabolism if very high doses, close to toxicity, were given. On the other hand, application of JH even if done repeatedly likewise did not stimulate, in fact it sometimes inhibited vitellogenin synthesis (Engels, 1981). The conclusion is that in honey bee queens JH is not controlling vitellogenin synthesis.

In all such experiments the queens have to be handled. It can be shown that this has to be done with extreme caution. A queen removed from her colony is probably as unhappy as her workers. Within a few hours a caged young queen for instance will drastically reduce the synthesis of JH, as can be demonstrated by the *in vitro* radioassay of the corpora allata. Similar effects are observed in young virgins if the rate of vitellogenin synthesis is stimulated with CO₂ treatment. A subsequent increase in vitellogenin production only occurs in queens introduced into colonies, not in caged queens (Engels et al., 1976). In order to overcome such difficulties, we developed an *in vitro* system for honey bee organ culture (Kaatz et al., 1985). The medium was composed of numerous compounds and tries to mimic hemolymph conditions. We hope to better understand fertility control for instance at the level of fat body activity under *in vitro* conditions. Distinct factors can be added or eliminated. Neither worker bees attending the queen nor other colonial circumstances have any influence on her physiology.

In fact in the *in vitro* system similar differences in the rate of vitellogenin synthesis, as have been described before under *in vivo* conditions, can be monitored. For instance if we compare fat bodys removed from egg-laying or non-laying queens. In both types of preparations, JH added to the medium likewise failed to stimulate vitellogenin synthesis. The *in vitro* studies therefore confirm the earlier experiments carried out with intact bees. But which factor or factors are then responsible for fertility control in honey bees? Recently it was found that head extracts can stimulate vitellogenin synthesis *in vitro* (Kaatz, 1987). Only extracts from egg laying queens seem to contain the active principle. As heat treatment inactivates the head factor, perhaps a brain peptide hormone is the candidate. But these preliminary data still have to be confirmed by further experiments. For the time being an interpretation based on the fact that in social insects such as honey bees the queen has to be able to produce eggs permanently with remarkable daily frequencies seems acceptable. Depending on colonial conditions a reduction of oogenesis within a short time as well as a sudden increase in oviposition may be necessary. Such

requirements demand a rapid modulation in the rate of vitellogenin synthesis which perhaps cannot be mediated by appropriate JH titer changes. The release of a stored brain factor presumably is instrumental in exerting such physiological control with higher effectiveness. In fact all the well documented examples of JH regulated vitellogenin synthesis concern non-social insects with cyclic egg production in which the control is effected according to a temporal pattern by induction (Dittmann et al., 1986).

What is the situation concerning stingless bees? No vitellogenin was detected in the hemolymph of young virgin queens during the first week after emergence. An increase in vitellogenin synthesis was recorded after the nuptial flight. Old stingless bee queens are physogastric, weighing about three times more than a young gyne. The egg laying queen produces vitellogenin at a high rate (Engels and Engels, 1977), a common feature in honey bees as well. Experiments concerning regulation of fertility are still lacking.

QUEEN DOMINANCE AND WORKER FERTILITY IN HONEY BEES AND STINGLESS BEES

One might assume that in the highly eusocial bees worker fertility would definitely be regulated by different physiological programs in the worker and the queen caste. This is, however, not the case. At least for honey bees it has been known for a long time that in queenless hives workers start the egg laying. Under queenright conditions worker fertility is inhibited by pheromone-mediated queen dominance. We were surprised to nevertheless find vitellogenin also present in the serum of such worker bees (Engels, 1972).

Looking for the vitellogenin titer in honey bee queens and workers of different age and function (Engels and Fahrenhorst, 1974), we could establish the following picture: Vitellogenin is already present in the queens hemolymph at the time of emergence. The vitellogenin titer increases rapidly in the young virgin where it amounts to 75% or more of all the serum proteins. Vitellogenin synthesis is stimulated immediately after the mating flights. This can be mimiced by CO₂ exposure (Engels et al., 1976). Later or when oviposition has started, the titer may decline somewhat due to drainage of yolk material into the oocytes.

In the worker bees of queenright colonies, the increase in vitellogenin titer is slightly delayed if compared with the queen, and the peak rarely exceeds 40%. Vitellogenin is found in all bees during the nursage age. In the nursing worker, vitellogenin is intensively synthesized although there is no vitellogenin uptake in the ovary. In *Apis* queenright workers vitellogenesis is completely suppressed. Accordingly vitellogenin production and incorporation are not linked together as has been described for many insects. Most of the newly synthesized vitellogenin disappears from the hemolymph within 1. or 2 days. Apparently the unused yolk precursor material is recycled in the fat body. It finally disappears in conjunction with the behavioral switch from hive to field duties in old foraging workers. Accordingly, in honey bees queen dominance does effectively prevent the workers from laying eggs. A closer look at the workers' physiological situation shows that vitellogenin production is reduced and restricted to the nurse age, and yolk is never deposited in oocytes.

Is this also applicable to the stingless bees? In the species we are mainly studying, *Scaptotrigona postica* from Brazil, under queenright conditions all the workers are to a limited extent fertile during the nurse stage. Then they have a high vitellogenin titer and lay some eggs: trophic eggs eaten by the queen or functional eggs developing into males. Stingless bee workers, therefore, are capable of producing two types of eggs, distinct in structure and destination. The increase in vitellogenin synthesis and

titer is observed about a week after emergence and lasts much longer than in honey bees, up to an age of 30 days of more (Engels and Engels, 1977). It has to be mentioned that the total lifespan is about 80 days. But the main difference is that also under queenright conditions all the workers incorporate vitellogenin into growing follicles and oviposit. Queen dominance in stingless bees evidently does not keep the workers at a really infertile status, though most of the workers' egg production is utilized for nourishment of the queen and not for their own offspring.

THE QUEEN-LESS WORKER, A QUEEN-LIKE FEMALE ?

What about worker bees devoid of any queen influence? One could assume that in queenless colonies the workers would perhaps become more queen-like. In fact in the case of orphan honey bee colonies some of the nurse bees do not become foragers. A few of these workers, when 15-20 days old, start egg laying. The vitellogenin titer in these egg-layers was found to increase and almost reach the level typical for laying queens. But the rate of vitellogenin synthesis and also the number of eggs produced by workers remains low. Other bees exhibit aggressive behavior towards the egg laying workers. Most of these non-laying bees were found to produce less vitellogenin. Therefore, the workers in queenless honey bee colonies develop various patterns of fertility, but normally differ from a real queen in many respects. The only known exception being the South African Cape bee, *Apis mellifera capensis*. Here single false queens occur in orphan colonies, surrounded by a court and behaving queenlike. Such an egg laying *capensis* worker is evidently much more a queen than in other races, and even by automixis is capable of producing female offspring, including queens. The physiology of these false queens still has to be studied.

In stingless bees the workers in orphan colonies continue to produce eggs, but not at an increased rate. Sometimes eggs abnormal in size and shape are laid. The physiology is yet unknown as well.

QUEEN INFLUENCES ON GYNE PRODUCTION, MAINTENANCE OF THE MONOGYNOUS STATUS

In the honey bee gyne production is normally controlled by the queen. Her dominance, operating through pheromone effects on the workers, prevents them from building royal cells and from supplying a queen larva therein with plenty of royal jelly.

In stingless bees there is no such rigid control of queen rearing. We have principally three modes of queen production. In some of the cluster nest building species - if the colony is growing fast and many cells are constructed at the same time - sometimes two of them may be very close to each other. Then a larva successively eats the provisions from these two cells and thereby becomes an enlarged queen. In the Trigonini big royal cells are permanently constructed in all queenright colonies. These queen cells receive much more provisions, again resulting in a large queen-biased larva. In the Meliponini a genetic background for queen formation has been proposed (Kerr, 1987).

If in a honey bee colony several queen cells are built at the same time, then the first gyne to emerge kills her younger sisters. Stingless bees have other mechanisms for eliminating supernumary queens. In *Scaptotrigona postica* for instance such moriture virgins are fixed and plastered with sticky cerumen. We may conclude that monogynous conditions can be achieved using many different mechanisms.

REPRODUCTIVE HOMEOSTASIS ON THE COLONIAL LEVEL

Consequently reproductive homeostasis at the colonial level in honey bees and stingless bees must be regarded today as being stabilized in a

manner which excludes the possibility that a worker can really function as a fertile queen. This is still feasible in sweat bees. If the functional queen gets lost, a worker will be mated and will replace the queen. Such replacement reproductives later on become fertile and queenlike (Michener, 1987). In eusocial societies reproductive homeostasis includes the regulation of gyne and drone production and survival. In addition to this, colony division by swarming - which in stingless bees requires the preceding foundation of a filial nest - has to be regulated. Although we are still far away from an understanding of all the equilibrium situations and behavioral shifts towards another homeostatic condition, it would seem that pheromonal messages, especially such released by the queen, are often involved in the regulatory system (Engels, 1987).

MALE ORIGIN, DRONE DEVELOPMENT, AND FEMALE DRONE CHARACTERS

The general Hymenoptera pattern of male determination in unfertilized eggs is extended in the eusocial groups by a possible dual origin of males: besides the facultatively uninseminated queen eggs, the obligatorily unfertilized worker eggs laid by unmated females also give rise to male production. Drone larvae regularly develop in worker larvae environment and receive worker-type larval nutrition. In stingless bees adult drones resemble workers more than queens (Kerr, 1987), but if occasionally a male larva receives queen provisions, a giant drone results (Imperatriz-Fonseca, 1976).

Recently a long standing presumption could be confirmed. Honey bee drones synthesize vitellogenin (Trenczek and Engels, 1986), a typical female-specific protein in most insect species. Probably the expression of this female character in a male genetic situation is connected with the unique circumstance that in the haplo-diploid Hymenoptera no sex specific genome elements exist. Female characters are evidently incompletely suppressed in male bees.

NUTRITIONAL BASIS OF CASTE DEVELOPMENT: SPECIAL QUEEN OR WORKER DIETS?

Let me turn now to the well known trophogenic basis of caste development. The search for a queen-determining factor was unsuccessful (Rembold, 1987). Instead of this the concept of a "nutritional balance" is being discussed today. But which factors are balanced? Food quantity, food quality, or both? In a series of experiments we modified diets for *in vitro* rearing of honey bee larvae in order to investigate this question. The main purpose was to compose a semi-artificial diet resulting in a worker-biased development (Wittmann and Engels, 1987). We found that the amount of larval food consumed by a female bee larva is not very significant for caste differentiation. When the food was made sweet, phagostimulated intake of large quantities of food-sap sometimes resulted in giant sized workers. Non-tasting food inhibited feeding, and consequently dwarf adults were the result, amongst them some pure queens. Food quality - which means richness in yet not fully listed essential factors - is apparently more important for caste development. Heat treatment of royal jelly, diets without yeast extract etc. decreased survival rates of the larvae, but increased the percentage of adult workers.

Therefore, the interpretation was that rich and surplus food supply - in honey bee queen cells always a considerable amount of royal jelly is left after the larva stops eating - shifts differentiation towards queen characters. On the other hand, a strictly balanced nutrition and a limited amount of diet results in worker development. In the eusocial honey bee apparently not a queen diet, but an economical worker diet evolved. From the standpoint of colony investment, worker brood consequently requires

minimal energy input. The reduced fertility of these females helps to avoid conflicts with the principle reproductive subject, the queen. Dietary worker caste formation is mediated by the provisioning nurse workers themselves and based on behavioral differences. In honey bees this is expressed in progressive and caste- and sex-specific nursing of the brood. In stingless *Trigonini* bees it is constituted by the supplying of future queen larvae with significantly more provisions.

ENDOCRINE CONTROL OF CASTE DIFFERENTIATION

Such trophogenic stimuli must doubtlessly result in a specific endocrine reaction; most probably in an increased production of JH during critical larval stages. Sensitive periods for application of external JH were described which cause worker larvae to differentiate into queens. Similar results have been obtained for both honey bees and stingless bees. But up to now no clear evidence was available on distinctly different titers which must be effected by this causing specific rates of JH synthesis in the respective larval stages. Hormone titer determination by radioimmunoassays or bioassays and the *in vitro* control of hormone synthesis clarified these points. Here I can report on recent results in stingless bees JH synthesis measurements by explanted copora allata in our new *in vitro* organ culture medium (Hartfelder, 1987).

The results clearly indicate a much higher JH production in the queen than in the worker larvae. This is also true after correction of the data in accordance with the larval body weight. But most impressive is the ratio of queen/worker JH synthesis per body weight equivalent. This ratio is between 1.5 and 2.0 throughout late larval and early pupal development. Only in spinning 5th instar larvae the quotient jumps up to 5:1. The future queen is producing much more JH exclusively in this period of late larval development. We think that the resulting high hormone titer switches differentiation of caste-specific characters in the course of the subsequent metamorphic differentiation to the queen gate. Intermediate JH titers presumably give intercaste morphs.

The nutritional stimuli provided by the queen-specific diet apparently induce an enhanced JH production during critical developmental periods. It will be interesting to check whether this is only true for queens in the highly eusocial stingless bees and honey bees, or also for females in solitary bees. Data on these developmental endocrine events would then facilitate a better understanding of the evolutionary changes which lead to queen or worker caste formation. Thus external stimuli trigger off in a yet unknown sequence of internal reactions the expression of caste syndromes in the eusocial bees. Morphogenesis is thereby controlled via caste-specific quantities of JH the acting morphogen.

CASTE SYNDROMES: POLYMORPHIC MODIFICATION OR GENETIC POLYMORPHISM?

The last aspect concerns the resulting caste polymorphism. In the discussion on socioevolutionary aspects, genetic polymorphism as a background for selfish or altruistic behavior is very often presupposed. Are there any indications that worker and queen characters - with regard to morphology and physiology - could also be realized through expression of different sets of caste-specific genes? In fact there is no evidence of this. The intercaste individuals seem to be more an indication of a modulation in caste differentiation resulting in graduated phenotype modifications. But the field of molecular genetics on the caste syndrome is as yet uninvestigated and is open for future research.

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SYMPOSIUM

Caste and Reproduction in Social Insects

Session I: Caste Differentiation

Session II: Reproduction

Organizers: Wolf Engels and Klaus Hartfelder

Introduction

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Caste development and control of reproduction in social insects are two sides of the same medal. Both result from the establishment and accumulation of successive differentiating processes in the ontogenesis of individuals living in a social environment. Under primitive social conditions the reproductive capacity of a female is largely defined by its position in a dominance hierarchy, and its caste status remains at a purely functional level. The stability of a social system based on such behavioral differences is rather low. Provided that a certain level of relatedness exists among the members of such a community and that external conditions are permissive, the trends for the transformation and fixation of behavioral differences into physiological and morphological caste characters would be predicted from the classical hypothesis of the kin selection theory as well as from its reformulation as an epigenetic theory (West-Eberhard, this volume).

From this stage onwards the sort of questions asked in studies on caste development and control of reproduction in social insects start to differ. The only aspect all these questions have in common is the elucidation of evolutionary and ecological constraints that hold each species at its respective level at social evolution.

In this symposium the influences of colony-intrinsic factors on caste development were pointed out by P. Lefeuve, Y. Roisin and J.M. Pasteels, Ch. Bordereau and S.H. Han as well as G. Bühlmann in their studies on termites, by W. Fortelius, E.L. Vargo and D.J.C. Fletcher, L. Passera, L. Keller and J.-P. Suzzoni in ants, and by M. Ono, M. Sasaki and I. Okada in the Japanese giant hornet. The role of extrinsic factors like temperature, photoperiod and habitat quality on caste differentiation and development of sexuals was investigated by D.A. Waller and J.P. La Fage, and by A. Luther in ants, and by G. Knerer in sweat bees. All these studies are concerned with the unveiling of feed-back loops at the colonial level almost extending down to the level of the individual. An example of this being the effects of photoperiod on voltinism and caste regulation in halictine bees.

At the level of the individual, the role of larval food in caste development was studied by M. Sasaki and T. Tsuruta, and by K. Bohrer and B.H. Smith in bees, while the function of trophallactic interactions between larvae and adult wasps was analysed by J.H. Hunt. As was pointed out by D.E. Wheeler, a trophogenic control of caste differentiation, i.e. a nutritional switch, apparently is not operative before the last larval instar, thus permitting the differentiation of only two caste morphs in most social Hymenoptera. Wherever the queen/worker switch was pushed to an earlier instar, a multiple caste system could arise. Besides termites, female ants are well known examples.

By dint of its morphogenetic function juvenile hormone (JH) plays an essential role in the differentiation of caste-specific characters during post-embryonic development. Due to its gonadotropic effects it can be regarded as endogenous correlate to dominance structures among members of many species of social insects. Ecdysteroid and JH titers in the hemolymph of pupal and adult honey bee queens were reported by H.H. Kaatz. Hypotheses on JH actions during caste differentiation in stingless bee development were reviewed by W.E. Kerr.

Important tools for our future understanding of the intricate mechanisms involved in JH dependent gene expression were introduced by G.D. Prestwich, S. Robles, M. Boehm and W.-S. Eng in their demonstration of JH-specific binding proteins in termite development. Interrelationships between caste specific differentiation in the endocrine system and the gonads in termites were discussed by W. Truckenbrodt. In highly eusocial bees, however, JH seems to play merely a minor role in the endocrine control of fertility since neither initiation nor maintenance of vitellogenin yolk protein precursor synthesis in the fat body seem to be JH-dependent. This has been pointed out by H.H. Kaatz and W. Engels. Honey bee vitellogenin is not a strictly female-specific protein as was shown by A. Zillikens and T. Trenczek who demonstrated its occurrence in haploid as well as in experimentally produced diploid drones.

Also at the behavioral level, JH applications did not cause significant changes in the dominance hierarchy in bumble bee colonies, as reported by A. van Doorn. Egg laying and ovipositioning strategies on the comb were discovered in the queen honey bee by R. Fleig, leading to a constant position of the dorsal side of the embryo pointing downwards, the significance of which is yet unknown.

To summarize: It is indeed fascinating to learn of recent investigations how similar the results of parallel socio-evolution turned out especially in several groups of social Hymenoptera. Apparently the female caste syndrome developed within the different taxa based on predetermined potentials common to all wasps, bees, and the ancestors of ants. In spite of the evolved convergencies in social life, it becomes increasingly evident that the complicated regulation of polymorphic caste formation and caste-specific reproductive capacities together with all the corresponding physiological and behavioral differences are principally controlled by the same instruments which are present and also already operating in solitary insects. The involvement of hormones and pheromones in social regulation and maintenance of colonial homeostasis is nowadays a well known confirmation of this hypothesis. Future research will contribute to a better understanding and a more complete knowledge of the evolutionary success and the enormous diversity of the social insects living amongst us.

The symposium consisted of two afternoon sessions chaired by Ch. Noirot, L. Passera, D.E. Wheeler and W. Engels, and a number of posters.

Replacement Queens in *Nasutitermes coxipoensis* Holmgren (Isoptera, Termitidae, Nasutitermitinae)

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Termite societies are normally headed by a pair of imaginal reproductives which have founded the colony after swarming. Following the death of the royal pair, the orphaned colony can only survive if replacement reproductives develop. The phenomenon is widespread in lower termites (Miller 1969). In higher termites, of which our knowledge is fragmentary, the replacement of the reproductive sexuals may occur in some species (Noirot 1956, 1969). The loss of the functional reproductives leads to variable results depending upon the different species. The replacement sexuals may develop from alates (adultoids), from advanced nymphal instars (nymphoids) or from worker (ergatoids) (Noirot 1969). Moreover, in certain species none of the individuals are capable of developing into replacement sexuals (Noirot 1969). In the phylogenetically advanced genus *Nasutitermes*, recent papers (Thorne 1984; Roisin 1986) show that closely related species develop different strategies for the replacement of reproductives. Therefore, it seemed interesting to study this phenomenon in *N. coxipoensis*, a monogynous species from Neotropics.

MATERIAL AND METHODS

N. coxipoensis Holmgren builds epigeous nests in savannahs around Kourou (French Guiana). Sixty-nine nests were opened before, during and after swarming period (nine did not contain reproductives). The queen was removed in sixty of them (eight nests did not survive the operation). The nests were reopened twenty-one, forty-two, fifty-six or eighty-four days after orphaning. All removed queens and all replacement queens were weighted to determine the rate of physogastric development. In order to know the exact composition of colonies, three nests were entirely dissected after alate flights.

RESULTS

The three nests entirely dissected after alate flights contained a population composed of between 60,000 and 280,000 individuals. Moreover, from one to four third-instar nymphs were found.

The following table summarizes our observations :

Number of opened nests	Queen weight (mg)X \pm SD	Days after orphaning	Nests with replacement queen	Replacement queen weight (mg)X \pm SD
5	139 \pm 77	21	0	—
10	111 \pm 47	42	4	13 \pm 4
9	112 \pm 54	56	5	18 \pm 17
28	145 \pm 52	84	14	77 \pm 48

N.coxipoensis appears exclusively monogynous. When replacement occurs, the removal of the primary queen leads to the differentiation of only one adultoid queen in a minimum of six weeks. The four replacement queens observed six weeks after orphaning were not physogastric. Eight weeks after queen removal neither eggs nor larvae were found in the nests which did not replace their queen. The brood found in colonies which had physogastric replacement queens suggests that these queens became functional egg-layers. Simultaneously, the development of physogastry was extremely rapid. Twelve weeks after orphaning, six from the fourteen replacement queens reached a weight equivalent to that of the removed queen. Considering the close correlation between queen weight and egg-laying rate as noticed in monogynous colonies of N.corniger (Thorne 1982, 1984) or in N.polygynus (Roisin 1986) we can presume that in N.coxipoensis a normal reproductive function was restored. In polygynous societies of Nasutitermes, replacement queens act together to restore a normal egg-laying rate after orphaning (Thorne 1982; Roisin 1986).

In N.coxipoensis, replacement sexuals are adultoid and may differentiate even outside of the normal alate maturation period. At least, the replacement sexuals probably originate from a few third-instar nymphs like those found in nests entirely dissected. The replacement of reproductives by nymphs present in the nest throughout the year also seems possible every time in Amitermes atlanticus (Skaife 1955). We postulate that it may have a reserve of replacement sexuals constituted of a very small number of individuals. Contrary to Kaloterms which invests in the differentiation of numerous sexuals to retain finally only one pair (Grassé and Noirot 1946), the strategy developed by N.coxipoensis appears very economic for the society.

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Species-Specific Responses to Orphaning in Neo-Guinean *Nasutitermes*

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The number and type of reproductives encountered in *Nasutitermes* nests vary according to the species. While certain species are monogynous (e.g. *N. exitiosus*, Hill, 1942, *N. novarumhebridarum*), high numbers of queens are common in others, such as *N. corniger* (Thorne, 1982), *N. princeps* or *N. polygynus* (Roisin and Pasteels, 1985, 1986b). Apart from normal imaginal reproductives, ergatoids (= worker-derived sexuals) are known in *N. columbicus* (Thorne and Noirot, 1982) and microimagoes (dwarf adults) in *N. princeps* (Roisin and Pasteels, 1985). Such differences are of special evolutionary interest as they involve closely related species with the same basic caste system. In this paper, we report and discuss the results of queen removal experiments carried out on three Neo-Guinean *Nasutitermes*: *N. princeps*, *N. novarumhebridarum* and *N. polygynus*.

METHODS AND RESULTS

The whole region of the queen cell was removed from nests of *N. princeps* and *N. novarumhebridarum*. Replacement reproductives (RR) were looked for after time intervals varying from 2 to 10 months. In *N. polygynus*, we removed all 5 nests of a polycalic system and collected the rebuilt nests 6½ months later. Samples of nymphs and alates were taken, when present, at the time of orphaning. Original and replacement reproductives were in most cases weighed and submitted to oviposition tests (Roisin and Pasteels, 1985).

In *N. princeps*, imagoes readily differentiated into RR in 12 cases out of 13. If no alates or late nymphs were present at the time of orphaning, a shortening of the nymphal development occurred, leading to microimagoes that took over the reproductive role. The number of replacement queens ranged from 8 to 180. At the colony level, normal egg-laying rates were in favourable cases restored after two months (Roisin and Pasteels, 1986a). In one instance, only one microimaginal queen was present 2½ months after orphaning, and numerous workers had undergone a beginning of sexual development, giving rise to intercastes (Roisin and Pasteels, 1986c). Whether these worker-derived individuals could ultimately have become functional RR is not known.

Seven nests of *N. novarumhebridarum* were re-collected. Five of them contained alates and/or late nymphs (4th and 5th instars) at the time of queen removal. In all the nests, RR developed from workers, not from nymphs or imagoes. The ergatoids began laying eggs after about 6 months. The two nests re-examined after 8 months contained, respectively, 9 and 34 ergatoid queens with swollen abdomens and several males; eggs and larvae were also present.

In *N. polygynus*, hundreds of young imagoes differentiated as RR in the queenless, rebuilt nests. Many of them showed signs of incomplete sclerotization, indicating an accelerated maturation (Roisin and Pasteels, 1986b).

DISCUSSION

Reproductive replacement by imagoes (N. princeps, N. polygynus) seems much more rapid than by ergatoids (N. novarumhebridarum). These interspecific differences can be related to the species' ecology. N. princeps and N. polygynus feed on diverse, often scattered sources of dead wood. Both species expand their territories and establish new societies by budding off new nests in which sexuals eventually differentiate. N. novarumhebridarum societies usually remain confined to one tree, on which they feed, and are headed by their founders. Ergatoids were never found under natural conditions. Colony-level advantages of replacing the sexuals quickly may thus not be essential for this latter species. At the individual level, N. princeps and N. polygynus alates can side-step the critical phases of swarming flight and incipient colony development by differentiating in a pre-established colony. The inability of N. novarumhebridarum alates to do so is puzzling, as adultoid differentiation is known to occur in a wide array of species belonging to all subfamilies of the Termitidae. One possible explanation is that in a species relying only on dispersal flight for colony multiplication, it may be adaptive for the sexual brood to resist any social perturbations caused by queen loss and proceed towards the alate, for example if queen death accompanies food exhaustion. For the workers, the ergatoid pathway would represent the last hope of reproduction.

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Soldier Regulation in the Higher Termites: Stimulatory Influence of the Royal Pair

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Soldier proportion in termite societies was shown in many species to be regulated by inhibitory processes, but it might also result from stimulatory mechanisms. We have studied the role of the royal pair on soldier formation in higher termites.

MATERIAL AND METHODS

Colonies of Cubitermes fungifaber (Termitinae) and Nasutitermes lujae (Nasutitermitinae) were collected in Ivory Coast. In both species, soldiers develop from workers. Soldier production was compared in homogeneous groups of 200 workers (N.lujae) or 300 workers (C.fungifaber) reared either in the presence or in the absence of the queen and/or the king.

RESULTS

In C.fungifaber, soldier production in controls is low ($0.06 \pm 0.042\%$) but the presence of the royal pair allows for a threefold increase ($0.175 \pm 0.034\%$). In N.lujae, the mean number of soldiers produced in the presence of the royal pair ($34.18 \pm 22.89\%$) is significantly higher than in orphan groups ($16.13 \pm 6.89\%$). The queen and the king have a stimulatory influence of the same order of importance. So, soldier production is strongly stimulated by reproductives in both species but great variations, apparently not related to a seasonal cycle, can occur between the different nests.

Considering the preponderant role of juvenile hormone in soldier differentiation, Lüscher (1975) assumed that the reproductives could induce soldier formation via a primer pheromone activating the corpora allata. However, our results suggest that the action of the reproductives could be prothoracicotropic as well as allatotropic.

Soldier proportion in termite societies probably result from the balance between the stimulatory action of the reproductives and the inhibitory action of the soldiers which has been shown pheromonal in nature in N.lujae (Lefeuve and Bordereau 1984).

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Caste Differentiation in Incipient Colonies of the Termite *Macrotermes michaelseni*

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Incipient colonies of the East African grassland termite *Macrotermes michaelseni* (Sjöstedt) were raised under various experimental conditions in the laboratory. Soil quality and size of the containers was varied, as well as the number of reproductives per group, the number of eggs or additional larvae. Under normal conditions, presoldiers are the first differentiated larvae to appear. If they are removed systematically, the colonies are capable to compensate. The additional presoldiers are likely to be recruited from the sterile female larvae which otherwise would have developed into minor workers. The balance between the two sterile sexes remains roughly constant, while the proportions of the two sterile female castes (minor workers vs soldiers) develop to the detriment of minor workers.

Social interactions such as grooming by parents or fosterers are crucial for survival. Composition and size of the group, speed of development of the young larvae and to some extent also the size of the container are responsible for determining the caste proportions of the young colony. Although it was not possible so far to localize a "soldier pheromone", the results can be best explained by such an agent.

Different Patterns of Female Behaviour in Mono- and Polydomous *Formica* Populations

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Secondary polygyny in ants is commonly considered a derived character evolved to meet some particular ecological conditions (Hölldobler and Wilson 1977). Variation in female behaviour might lead to polygyny and polydomy, through the evolution of different reproductive tactics (Rosengren and Pamilo 1983). Gösswald and Schmidt (1960) report variation in dealation and mating behaviour of mono-, oligo- and polygynous *Formica* s.str. ants.

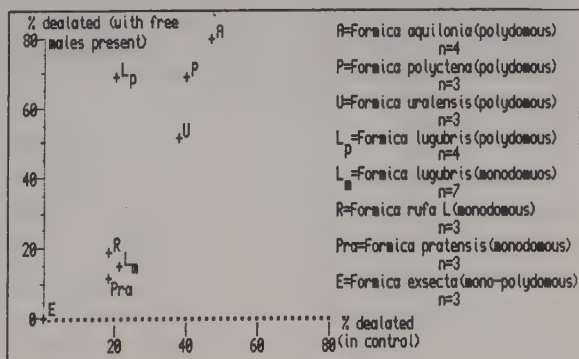
The present paper summarizes results of studies on dealation, mating and diel activity in females from monodomous (presumed to be mainly monogynous) and polydomous (polygynous) *Formica* populations. Seven species from Finland and one from Switzerland (polydomous *F.lugubris*) were studied (Fig. 1).

METHODS AND RESULTS

The dealation process was studied in relation to four laboratory conditions; control, CO₂-narcosis, presence of free conspecific males and presence of encaged conspecific males (prevented from physical contact with the females).

The dealation rate is higher in females from polydomous than in females from monodomous populations (Fig. 1). Fig. 1 also demonstrates a clear difference in the dealation response to free males (due to different mating tendencies, below) as well as the total absence of dealation in *Formica exsecta*. Further analysis of the material (excluding *F.exsecta*) reveals that no dealation occurred during the first week of the experiment in females from monodomous populations while the dealation was frequent throughout a three-week period in polydomous females. Indications of bimodality with maxima at the beginning and the end were also observed in the latter case. CO₂-narcosis induced a significant increase of the dealation rate in all the females (regardless of origin) while encaged males did not affect the dealation in any observed case.

Fig. 1. -- Percentage of dealated females in the free-male chamber (see text) versus the corresponding percentage in the control chamber. n=number of each chamber category.



Mating in laboratory-conditions was studied in the free-male chamber (above). True copulae were seen only between ants from polydomous populations. Avoiding of males was the rule in females from monodorous populations but was also seen in some of the polydomous females. Examination of the spermathecae confirms that even in the presence of males virtually all females from the monodorous populations stayed virgins while about 50% of the females from the polydomous populations were inseminated. Note that none of the polydomous females from the non-male chambers were fertilized.

When mounds of polydomous *F.aquilonia* were excavated at the end of the nuptial flight period, dealated females (according to ovarian state) were often found in clusters, 20-30 cm inside the mound. The same mounds generally had alates on or near the surface. 83% of the dealates had living sperm in the spermatheca while only 3% of the alates were inseminated.

Diel activity of polydomous (*F.aquilonia*) and monodorous (*F.rufa*) females was tested in an automatic activity recorder based on infrared-sensitive phototransistors. In both cases activity was light-regime-dependent. The activity of monodorous females was restricted to a few hours in the relative foremidday. In the polydomous females activity was recorded throughout the enlightened period, although a clear peak of high activity was seen in the relative morning.

DISCUSSION

Gösswald and Schmidt (1960) claim that accumulation of fermentation products affects the dealation of *Formica* ants, probably to a higher extent than e.g. pheromones. This is supported by the findings that CO₂-narcosis but not the presence of (encaged) males increase the dealation rate.

A diverse behavioural pattern was found in the polydomous (-gynous) females as compared to the more stereotype behaviour of the monodorous (mono-oligogynous) females. It is tempting to apply these results to the model for maintainance of polygynous/polydomous populations based on frequency and density dependent selection between dispersing and nondispersing females, proposed by Rosengren and Pamilo (1983).

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Queen Control over the Production of Sexuals in the Fire Ant, *Solenopsis invicta*

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A fundamental feature of hymenopteran societies is the ability of queens to control reproductive development in nestmates (Wilson, 1971, Fletcher and Ross, 1985). Knowledge of the means whereby this control is achieved in different species is important to our understanding of the evolution and maintenance of sociality in insects. However, the mechanisms used to effect queen control in most species remain poorly known. This is particularly true of the highly eusocial species, where, unlike the primitively eusocial taxa, queen control appears to be achieved primarily by non-overt means. The aim of this work was to determine whether queen control over the production of sexuals occurs in the fire ant, *Solenopsis invicta*, a highly eusocial species, and, if possible, to shed light on the mechanisms involved.

EXPERIMENTS AND RESULTS

S. invicta occurs in two distinct forms in the southeastern U.S. In the monogyne (M) form, colonies possess a single functional queen, while colonies of the polygyne (P) form contain many functional queens. Observations of several populations over the entire range of this ant suggested to us that P colonies produce far fewer sexuals than colonies of the M form. To determine whether this was in fact the case, 25 large colonies of each form were collected in spring, and again in summer and fall. The ants were taken to the laboratory where all sexuals (alates, pupae, and discernibly sexualized larvae) were counted. The total numbers of sexuals in colonies of the M form far exceeded those of the P form for each of the three seasons (Vargo and Fletcher, 1986a). This was also true for the number of females during two of the three seasons and for the number of males during all three seasons. These results led to the working hypothesis of an inhibitory effect on the production of sexuals associated with high queen number.

To test this hypothesis, we divided P colonies to correspond to natural M and P colonies. The workers and brood from 25 colonies were divided into equal halves, with one half (M) given one of the original queens, while the other half (P) received all the remaining queens (range=24-186). Discernibly sexualized larvae (>3 mm long) appeared within 48 h in many of the M halves. After six weeks all of the M halves had produced large numbers of both male ($\bar{X} \pm SD = 256.3 \pm 60.4$) and female (139.8 ± 54.2) sexuals, while only one of the P halves had produced any (3 females and 1 male; Vargo and Fletcher, 1986b). Similar results were obtained when 7 colonies were divided into queenless (Q-) and P halves, indicating that eggs and larvae capable of sexualization are present in P colonies but are normally prevented from developing into sexuals. Evidently, in response to high queen number, female larvae are fed less and develop into workers. In contrast, male larvae must be killed since they have no alternative line of development.

Inhibition of the production of sexuals in P colonies is consistent

with two hypotheses: 1) differences in worker/larva ratios associated with queen number; 2) pheromonal queen control. To test these hypotheses, it was necessary to develop a relatively small bioassay unit. This was achieved by drawing 3 10-g units of workers and brood from each of 11 colonies. Units received 20 queens (P treatment), one queen (M treatment), or no queens (Q- treatment). Alates were counted and removed weekly until all sexuals had eclosed from the Q- units (5-6 weeks). There were significantly more male and female sexuals produced in the Q- treatment than in the M and P treatments (Vargo and Fletcher, 1986c). These results demonstrated that small experimental units are capable of producing sexuals and are sufficiently sensitive to differences in queen number to use them for testing hypotheses concerning the role of queens in regulating the production of sexuals in *S. invicta*. Similar experiments showed that even smaller (5-g) units could be used.

To determine whether differences in worker/larva ratios were involved in this inhibition, more eggs than could be laid by a single queen in 24 h (ca. 1500) were added daily to Q- 5-g units (n=8). These units produced more sexuals than similar units containing one queen, but did not differ statistically from Q- controls which did not receive eggs (Vargo and Fletcher, 1986c). These results rule out the possibility that differences in worker/larva ratios significantly affect the production of sexuals in such experimental units. They also exclude the possibility that chemical cues are transmitted by the queens via their eggs.

To test the hypothesis that queen pheromones are involved, 10 freshly killed corpses of functional (egg-laying) queens were added daily to Q- 5-g units (n=20). These corpses inhibited the production of sexuals compared with Q- controls which did not receive corpses, but not as effectively as did living queens (Vargo and Fletcher, 1986c). On the other hand, corpses of alate virgin (non-egg-laying) queens with wings removed did not inhibit the production of sexuals.

The addition of living queens to previously Q- units (n=7) in which large sexual larvae had developed resulted in the execution of many of these larvae by workers (Vargo and Fletcher, 1986c), indicating that queen control over the production of sexuals can act retroactively after larvae are sexualized. A similar but less pronounced result was obtained by the addition of functional queen corpses, but not alate virgin queen corpses.

These results provide evidence that functional queens exert control over the production of sexuals in *S. invicta* through pheromones that influence the behavior of workers toward both male and female larvae.

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Male Differentiation in the Argentine Ant *Iridomyrmex humilis* (Mayr)

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Considerable studies have been devoted to female caste determination in ants (Passera 1984). On the other hand the factors regulating male production remain to be studied. A better understanding of the appearance of males may enable us more effectively to control pest ants such as the Argentine ant.

This species has invaded the French Mediterranean coast for fifty years. It shows a high degree of polygyny and because the workers are unable to lay reproductive eggs, all the males are the progeny of mated queens (Markin 1970, Benoist 1973). According to these authors, the occurrence of males in field nests is rather long lasting. Another salient point is the lack of nuptial flight.

The objective of this work is to document the conditions of males production in the laboratory.

Materials and Methods

Large polygynous societies of Argentine ant were collected by digging the sand and reared as stock colonies in the laboratory. For experimental cultures, large societies were split in standard units. Large societies and standard units were housed in plastic boxes of suitable size. Both were fed sucrose solution and artificial protein diet.

Results

In stock large societies males are reared at periods of 3 or 4 months. In the meantime broods transferred from these societies to standard queenless or queenright (1 to 5 queens) units, develop males. A reasonable explanation is that haploid eggs are laid by mated queens all the year round so the rearing of males is always possible; it is in striking contrast to the other ants where it is generally assumed that virgin eggs are laid seasonally. But why is the development of these eggs not a continuous phenomenon but a cyclic phenomenon?

Comparative experiments suggest that success in the rearing of males as far as the pupae stage depends on parameters which are connected.

. Ratio workers/larvae: We have recorded males production in standard units where the ratio workers/larvae ranged from 0.25 to 25 (fig.1). The higher the ratio is, the higher is the number of male larvae.

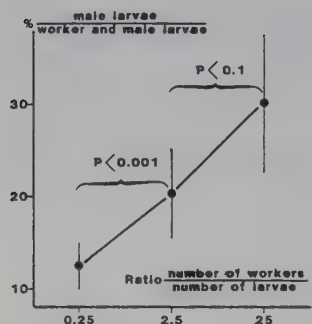


Fig.1. - Male larvae production in standard units where the ratio number of workers/number of larvae range from 0.25 to 25.

. Queen control: In standard units where the ratio workers/larvae were high the presence of queens was not an obstacle to the rearing of males (fig.2). These data suggest the

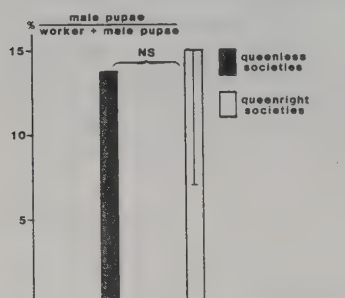


Fig.2. - In standard units where the ratio workers/larvae is high (13.75), there is as much male pupae in queenright societies as in queenless societies.

lack of a queen inhibitory pheromone controlling male differentiation. However in large societies where broods are very numerous if queens were removed the rearing of males begins again. These data support evidence that the queens act by means of appropriation of food.

. Diet: In experimental societies deprived of protein food male larvae fail to pupate.

So the differentiation of males seems principally controlled by the amount of available food.

In discussion we pay special attention to the above parameters in nature. In addition we discuss the benefit versus cost that permanent presence of male larvae involves.

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Reproductive Biology of the Giant Hornet, *Vespa mandarinia* Smith

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Although some mating behavior in vespine wasps has been reported (Batra, 1980, Ross, 1983, Matsuura, 1984), information on its pheromonal regulation is so far very limited. The present paper describes the reproductive strategy of the giant hornet, *Vespa mandarinia* Smith.

1. Mating behavior in the vicinity of nest entrance

Many drifting males were attracted to the vicinity of the nest entrance of an underground nest. When new queen left the nest, the attracted male(s) tried to copulate (Fig.1). In forty-five matings that were observed around the nest entrance, most of them were accomplished on the ground. The new queen stiffened when the male grasped her thorax, and accepted his copulatory attempt. The distance from the nest entrance to the mating site ranged from 0 (inside the nest) to 650 cm (av. 119 ± 164 cm). The average duration of copulation was 20 ± 7 s (10 to 26 s). Mating occurred mainly between 10:00 and 13:00. The timing of the male aggregation and the queen leaving the nest was well synchronized. Dual mating was infrequently observed in the field. Under laboratory condition, both males and new queens were capable of multiple mating.

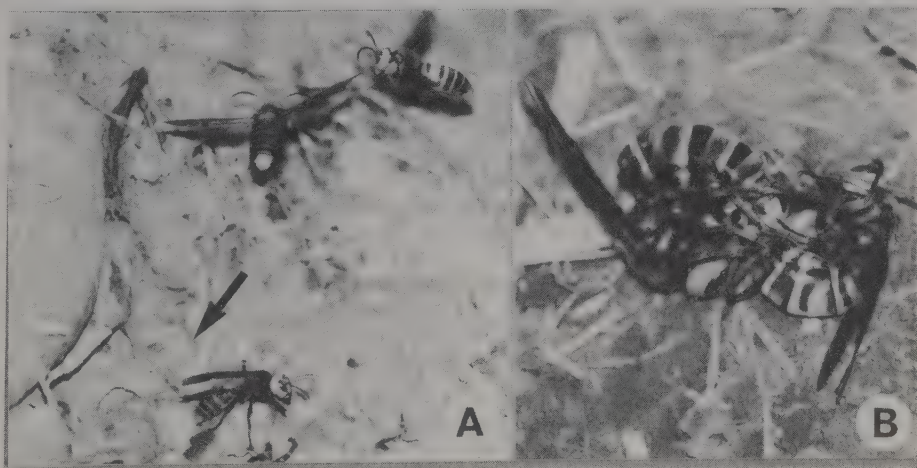


Fig. 1 -- Mating behavior of *Vespa mandarinia* Smith

- A Males attracted to the nest entrance (arrow)
- B Copulation (left: new queen, right: male)

2. Role of aggregation and sex pheromones

The mating behavior of V. mandarinia is explained by two sequential steps which are regulated by pheromones: 1) attraction of many drifting males to the nest entrance by an aggregation pheromone secreted from all three castes, and 2) copulatory behavior by the attracted males with a new queen, which is guided by a queen-specific sex pheromone (Ono *et al.* 1985).

(1) Adaptive significance of aggregation pheromone in workers

The first evidence of aggregation pheromone in workers was shown by Okada (1961). Although the pheromone was extracted from workers and a positive biological activity was demonstrated, the adaptive significance of the pheromone has not been specified.

One of the most important roles of the pheromone is to attract many males to the vicinity of nest entrance as the mating site. The phenomenon whereby workers contribute to the chance of new queens mating by producing a pheromone should be of distinct adaptive significance because they are "genetic relatives".

The aggregation pheromone activity in workers has so far been determined only in V. mandarinia out of six sympatric species of Vespa in Japan.

(2) Queen-specific sex pheromone

The presence of sex pheromone in V. mandarinia, which releases the mating behavior of males has been demonstrated and the activity is queen-specific. When the sex pheromone (a fraction of queen extracts) was applied to the thorax of anesthetized workers, males attempted to copulate and mounted her. Before separation of the couple, the worker began nibbling the dorsal surface of the male gaster. In contrast to the aggregation pheromone, interspecific cross activity was observed in the sex pheromone among Japanese sympatric species of the genus Vespa.

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Temperature-Induced Soldier Differentiation in *Coptotermes formosanus* Shiraki (Rhinotermitidae)

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The mechanisms that regulate termite soldier-to-worker ratios are largely unknown. Soldier production often varies seasonally with new soldiers appearing during alate development (1, 3). Termites with warm-season alate flights could coordinate defensive and reproductive caste development by producing more soldiers at high temperatures. We examined soldier induction at different temperatures in *Coptotermes formosanus* Shiraki, a subterranean rhinotermitid that disperses alates in spring and summer in Louisiana (USA). We also examined seasonal foraging activity in Lake Charles, Louisiana.

MATERIALS AND METHODS

Termites were collected from baldcypress trees (*Taxodium distichum* (L.) Rich) in Lake Charles. In Experiment 1, we confined 200 *C. formosanus* workers with decayed baldcypress wood in screw-top glass jars with vermiculite and deionized water on February 20, 1986. Termites were incubated at 30°C and 25°C for five weeks (six colonies, two replications per colony per treatment). In Experiment 2, termites were kept at 31°C and 27.5°C and fed baldcypress (five colonies) or pine (*Pinus* sp.) (four colonies) for five weeks beginning June 21, 1986. After the experiments, we counted surviving workers, % soldiers produced (presoldiers and soldiers), and measured amount of wood consumed, and dry weights of five groups of 10 termite workers per jar.

RESULTS

In Experiment 1, significantly more soldiers were produced at 30°C than at 25°C (3.1 ± 2.3 SD vs 0.5 ± 0.4 SD) ($p < .001$), although survivorship was similar ($p > .05$). Termites ate significantly more wood ($p < .01$) and gained more weight ($p < .001$) at 30°C.

In Experiment 2, more soldiers were produced at 31°C than at 27.5°C (31°C baldcypress: 5.1 ± 4.1 SD, 31°C pine: 5.2 ± 2.9 SD, 27.5°C baldcypress: 2.1 ± 2.1 SD, 27.5°C pine: 2.9 ± 1.4 SD) ($p < .001$), but survivorship was similar ($p > .05$). Soldier production was not different on the two wood species ($p > .05$). Termites ate more wood at 31°C ($p < .001$), and consumed more baldcypress than pine ($p < .001$). Termites did not differ in weight according to temperature ($p > .05$) but gained more weight on pine than on baldcypress ($p < .001$). In both experiments, termite colonies varied significantly in soldier production ($p < .001$), survivorship ($p < .001$), wood consumption ($p < .001$) and dry weight ($p < .001$).

Field collections of repeatedly sampled colonies (RSC) and newly sampled colonies (NSC) on different collection dates in Lake Charles are shown in Table 1. Termite numbers and percent soldiers in traps (plastic pipe filled with cardboard and attached to host

trees) fell in winter and rose in spring. Differences among dates were significant for RSC traps but not for NSC traps.

Higher temperatures might trigger soldier differentiation via increased food consumption. Good nutrition influences soldier determination (5), possibly by augmenting juvenile hormone (JH) titres. It is well established that JH promotes soldier differentiation in both lower (4) and higher (6) termites. As soldier numbers rise, maturation of new soldiers is inhibited (2). Mortality or cannibalism could reduce soldier numbers in winter. Thus seasonal cycles in soldier ratios may be generated by temperature-sensitive soldier differentiation.

Table 1. Mean (\pm SD) number of termites and % soldiers (S) in traps on different dates in Lake Charles. Number of colonies sampled is in parentheses.

	8 Oct 1985	27 Nov 1985	18 Feb 1986	22 Apr 1986
RSC traps				
Termites	6949 \pm 5701 ^a ¹	2660 \pm 1662 ^b	2277 \pm 1351 ^b	5203 \pm 1221 ^{ab}
%S	3.7 \pm 4.5 ^{ab}	3.0 \pm 2.3 ^{ab}	10.1 \pm 5.1 ^c	4.4 \pm 3.4 ^{bc}
	(8)	(8)	(8)	(7)
NSC traps				
Termites	4219 \pm 4011 ^a	3700 \pm 3713 ^a		6002 \pm 3738 ^a
%S	12.2 \pm 11.4 ^a	5.3 \pm 3.6 ^a		9.8 \pm 9.0 ^a
	(9)	(9)		(5) ²

¹ Means followed by the same letter in rows are not significantly different at $p < .05$, Tukey's Studentized Range Test

² Traps collected 22 Apr or 5 May 1986

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Production of Sexualls in Relation to Nest-Condition and Habitat Quality in *Formica aquilonia* Yarrow (*Formicidae*, *Hymenoptera*)

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Among polydomous ant-species only a certain part of the nests produce winged sexualls. According to Herbers (1979) the sex-ratios vary at random and cannot be seen as a general optimal response to selective pressure. The following study suggests a relationship between habitat and nest quality, production of sexualls and sex-ratios.

METHODS

The work was conducted in 1982-86 on four polydomous colonies of *F. aquilonia* -- the most common species of red wood-ant in Finland. Two of the colonies are situated in woodland of stable quality, two in disturbed habitats (an urban area and a larch plantation). Most of the work was carried out in one of the stable colonies -- an area in Åker, west of Helsinki, with over 70 nests in an area of only 1.5 hectares. The volume of the living part of the nests was measured, as was also the nest temperature during the active period. The production of sexualls was determined by sampling and examining pupae (20/nest/year).

RESULTS

(1) Nests producing sexualls differ from nests producing only workers in average volume -- 176 litres compared to only 71 litres -- and are also significantly warmer during the active period, the difference during the rearing of the sexualls being 7-10°C.

(2) In the areas representing original woodland, the percentage of nests producing sexualls was 37-54%, in the disturbed colonies 12-21%.

(3) In the colonies with a high ratio of nests producing sexualls, the colony sex-ratio was strongly female-biased. Table 1 shows the ratios (a) as numerical rates, (b) as investment rates, based on preliminary calorimetric measurements of energy contents in sexualls (male/female = 0.59).

(4) The colony sex-ratio seems to be relatively stable from year to year even if the ratios in individual nests vary. In the Åker colony, the proportion of males in old declining was significantly (0.1% level) higher than in young prospering nests.

DISCUSSION

In the polygynous *F. aquilonia* hundreds of functional queens coexist in a nest and the conditions of relatedness between the workers are not necessarily those on which the theory of gyne-worker-conflict (Trivers & Hare, 1976) is based. The suggested relationship between the production of sexualls and habitat quality could be regarded as a strategy for maximizing the resource utilization in different or changing environments.

Table 1 Sex-ratios in Four Colonies of Formica aquilonia in 1982-86

Area & Year	Numerical Rate	Investment Rate	n	N
Åker 1982	0.16	0.09	49	18
Åker 1983	0.24	0.14	72	27
Åker 1984	0.19	0.11	72	34
Åker 1986	0.20	0.12	72	33
Träskända 1982	0.10	0.06	68	29
Träskända 1984	0.11	0.06	150	63
Träskända 1986	0.09	0.05	121	40
Klovis 1982	0.35	0.21	14	3
Brunakärr 1983	0.60	0.35	50	6

n = examined nests N = nests producing sexuals

F. aquilonia inhabits great uninterrupted areas of woodland where the colonies slowly spread by budding. The mated females mostly return to their mother-nest -- either to start egg-laying there or to be moved to new bud-nests by workers. In such habitats a female-biased sex-ratio could be a way of securing further budding.

In a poor or declining habitat, female-biased sex-ratios might be too energy-consuming, whereas the production of males can be regarded as a way of allocating the collected energy into cheap dispersing units. In Formica exsecta two male-morphs, micraners and macraners, probably representing dispersers and home-stayers are found (Fortelius et al., 1986). Similarly, a small ratio of males in a prosperous colony might secure its continued existence, while dispersing males from poorer areas provide the required genetic variation.

The material from the less female-biased colonies is still very small and can be regarded with scepticism. The material as a whole should, however, be enough to show that the sex-ratio for this species does not vary at random.

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Photoperiod as Cue for Voltinism and Caste Regulation in Halictine Bees

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Halictine bees not only exhibit remarkable diversity in the level of social behaviour, but they also show a range of voltinism in solitary species. The latter are mostly univoltine, although bivoltine and trivoltine species are known. A similar situation exists in social halictines where one, two, three or more broods of workers are produced before a final generation of males and queens appears. These differences in the life cycles could be either genetic, when they would not respond to extrinsic factors, or the environment could have a modifying influence. Field studies point to the latter situation: *Evylaeus malachurus*, for example, has one worker brood in England, two in central Europe and three in southern France. A cold spring and summer reduced the usual two worker broods to a single one in Germany 1984, while an excessively wet spring in 1975 obliterated the third worker brood in the Dordogne. It appears that caste initiation is under environmental influence, probably changes in the day-lengths. Subsequent experiments with different photoperiods during the nesting phase of *E. malachurus*, *E. linearis* and *E. cinctipes* resulted in the production or suppression of a first worker brood, depending on the day-length given. Spring-like photoperiods cause the overwintered queens to construct a nest with small cells in which they rear small workers. Summer-like photoperiods, on the other hand, result in the construction of big cells with large pollen on which queens develop.

Role of the Physical Property of Royal Jelly in Queen Differentiation of Honeybees

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In artificial rearing of queen honeybees, worker and/or intermediates frequently emerge even under the same conditions. The fluidity of royal jelly (RJ) has a critical role in the phenomenon.

Materials and Methods

Newly hatched (<24 h) larvae of *Apis mellifera* were used for all rearing. RJ used for both rearing and fractionation was collected 48 h after grafting. Food RJ was renewed every 24 h according to Sasaki and Okada (1972). Rheological measurements were made with a Creep meter (RE3305, Yamaden Ltd.) and controlled stress rheometers (Series III, Deer Ltd. and CS Rheometer, Carri-Med Ltd.).

Results and Discussion

1. Parallel experiments of rearing and measurement of RJ viscosity

The fresh RJ was prerequisitely exposed to 34°C, RH97% for 0 to 96 h, and subjected to rearing assay and rheological measurement. The RJ viscosity increased without loss of moisture under the "hive condition". A clear negative correlation exists between the viscosity and larval development; 30% (n=27) of larvae developed to adults with intact RJ (viscosity=0.3 x 10⁵Pa.s) but no adults emerged (n=20) with 48h-exposed RJ (1.0 x 10⁵Pa.s). Simple viscosity was indicated here as the criterion of the physical property because the elasticity component is very limited.

Fig. 1 shows other examples of treated RJs. RJ with 10% sucrose is about 50% less viscous than intact RJ; with 40% sucrose it is about 5 times more fluid. In bioassay of these two RJs, the average weight of mature larvae increased to 191 and 254 mg, respectively. The emergence rate also increased to 94% in the 40% sucrose group. Defatted RJ (EE RJ) also showed low viscosity and good development. In rearing on RJs of various lipid contents, no queen was obtained from intact RJ (15% lipids), whereas in the 1/5 lipids group, the average mature-larval weight exceeded 310 mg and 60% emerged as complete queens (Yoshida *et al.*, 1984).

The queen inducing effect of added sucrose seems to result from joint phagostimulatory effect and reduced viscosity. The importance of the latter was evaluated by using galactose. Despite the lower phagostimulatory activity and poor nutritional value, RJ with 10% galactose gave comparable results to RJ with sucrose, glucose, or fructose.

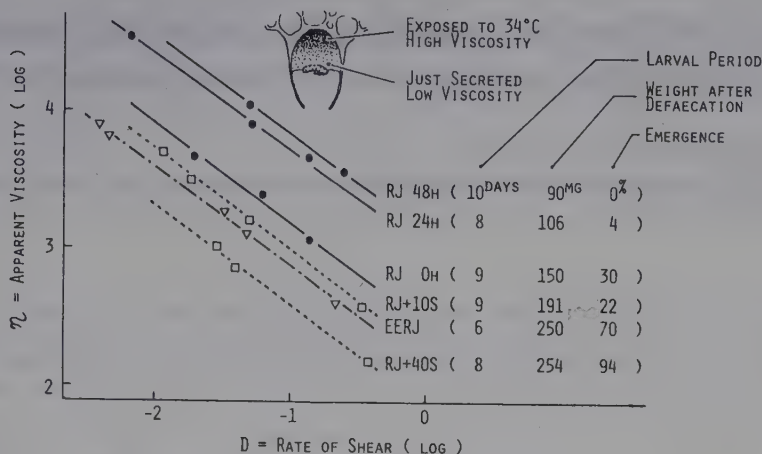


Fig. 1. Changes in the viscosity of some treated RJs and summary of the developmental criteria.

2. Origin of the viscosity: A simulation

Fractionation and recombination of RJ showed that an interaction between water-soluble protein and 10-hydroxy-2-decenoic acid was the main factor in the loss of fluidity and associated whitening of RJ. Low temperature and addition of sugar delayed the interaction. The soluble protein itself solidified at under 34°C, but is less important.

3. Behavioral aspect of feeding and possible mode of action

The behavioral repertoire of young "C"-shaped larva is very limited. They only turn slowly (60 to 120 min/turn) in the horizontal surface of RJ. The feeding behavior is exceptionally passive and seems to result in partial starvation in laboratory rearing. This probably inhibits the queen-specific function of corpora allata and produces various intercastes. The effectiveness of independent factors such as frequent renewing or stirring of RJ, high water content, low lipid content, and sugar addition, all of which are reported to promote queen induction, is explained by the improvement in the physical properties of the RJ. In nature, however, larvae are always provided with newly-prepared, fluid RJ until the queen cells are sealed, and no starvation occurs.

In mature larvae, active three-dimensional turning is released (10 to 20 min/turn) and old, solidified RJ can be freely ingested.

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Analysis of Free Fatty Acids in the Larval Provisions of the Halictine Bee *Lasioglossum (Evylaeus) malachurum*

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Many species of bees have been shown to include free fatty acids (FFA) in their larval provision in order to boost their nutritional quality. In the bee family Halictidae, several investigations have outlined the importance of cell size, the amount of provisions and temperature on the size of the emerging bees. However, to date no one has investigated the role of FFAs in larval nutrition in this family of solitary, communal, and social bees. We therefore undertook to describe the FFA content of larval provisions in the social halictine bee *Lasioglossum malachurum*.

MATERIALS AND METHODS

Pollen provisions were excavated from nests near Tübingen in Southern Germany in May and June, 1986. Pollen was collected from flying workers approx. one week prior to excavations. Additionally, nectar and cell linings were analysed. Pollen and provisions were first weighed and then extracted in spectral grade hexane. FFAs were then separated from lipids by the McCarthy-Duthy method and the former were derivatized with diazomethane prior to GC and GCMS analysis with a carbowax column.

RESULTS AND CONCLUSIONS

We have identified seven FFAs in pollen collected by the workers. The acids are: hexadecanoic, octadecanoic, octadecenoic, and two isomers each of octadecadiene- and trieneoic. Exact identifications of the stereochemistry have not yet been made. However, differences exist between the FFA composition of incoming pollen and that found in the provisions. The second isomers of the doubly and triply unsaturated acids were not found in the latter extracts. Additionally, approx. 1 µg per gram pollen occurs in pollen extracts while 10 µg occur in provision extracts.

Addition of Dufours gland secretion could account for this excess of FFA in the provisions. The same acids found in the provisions are also found in the gland. However, the mixture of FFA in the gland is heavily biased toward octadecenoic acid, whilst the mixture in provisions is biased towards octadecatrienoic acid. We therefore cannot rule out the possibility that acids are added to the provisions by the bees and then modified through enzymatic action. Alternatively, acids may be added or modified through the action of the bacterial fauna in the bee nests.

Variable Trophallaxis in Larvae of the Paper Wasp Genus *Mischocyttarus*

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Hunt *et al.* (1982; *Evolution* 36:1318) hypothesized that larval trophallaxis in social Vespidae has its evolutionary foundation in the similarity between larval saliva and floral nectar as nourishment for adult wasps. While this view is still believed to be correct, two issues were not addressed by Hunt *et al.*: 1) why is the concentration of nutrients so much higher in larval salivas than in floral nectars, and 2) what adaptive benefit accrues to larvae from the surrender of saliva to attending adults? The discovery of variable trophallaxis in *Mischocyttarus* suggests an hypothesis that addresses both of these questions.

The social wasp genus *Mischocyttarus* is exclusively New World and largely tropical in distribution. *Mischocyttarus* species are similar to the more commonly known paper wasp genus *Polistes* in both nesting habits and social biology. Final instar *Mischocyttarus* larvae, however, may readily be distinguished from those of all other wasps by the possession of a 1-, 2-, or 3-pointed lobe on the venter of the first abdominal segment.

A large, post-emergence nest of *M. immarginatus* was collected in Guanacaste Province, Costa Rica, on 5 February 1984. When samples of trophallactic saliva were collected from final instar larvae (by the technique of Hunt *et al.*, 1982) it was discovered that some larvae refused to surrender saliva. Refusal was accompanied by a remarkable behavior in which a larva withdrew its head sharply into its cell. The head retraction caused the abdominal lobe to rotate anteriorly and to become turgid and erect in the center of the nest cell. Field study on *M. immarginatus* in June, 1984, revealed that the lobe erection behavior is apparently a correlate of colony developmental state. Lobed larvae in pre-emergence nests always give saliva and never erected the lobes; some larvae in post-emergence nests refused to surrender saliva and erected their lobes.

An hypothesis is proposed that larvae in pre-emergence nests may surrender saliva when the level of colony nourishment is low in order to forestall their potential cannibalism by attending adults. Larvae in post-emergence nests, when nourishment levels in the colony are higher, may retain saliva at low risk of being cannibalized. If true, this hypothesis both ascribes adaptive value to larvae for saliva donorism and simultaneously suggests a scenario for directional selection to yield the very high nutrient concentrations known to occur in larval saliva. The pattern of withholding saliva as a correlate of colony nourishment further suggests a role for trophallaxis in the trophic determination of caste in social vespid wasps.

Evolution of Physical Castes and the Physiology of Caste Determination

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The origin of differences between physical castes in social insects must lie in the larval stage, when developmental patterns that produce alternate forms can be initiated. The process of caste determination comprises the effects of numerous social and physical factors on larvae.

Factors that affect caste determination during larval development fall into three categories: nutrition, caste pheromones, and physical factors. Nutritional variation can be traced to either availability of food in the environment or to its distribution by nurses. Pheromones act on larvae to inhibit development of a specific caste. The primary physical factor known to affect larvae during the process of caste determination is temperature.

Different systems of caste determination stress different mechanisms of control. For example, dependence on nutrition is seen in honeybees. In termites, larvae are more self-sufficient than in Hymenoptera, and their diet cannot be so easily controlled by colony members. Caste determination in termites therefore relies more heavily on inhibitory pheromones (reviews in Wilson, 1971). In north temperate climates, winter temperatures can be a major factor in queen determination (Brian, 1980). In the ant, *Plagiolepis pygmaea*, all three factors come into play during queen determination. To develop as queens, larvae first must meet overwintering requirements, then escape the inhibitory queen pheromone, and finally, fill their nutritional needs (Passera, 1969). A similar pattern holds for *Myrmica* (Brian, 1980), except that no effect of a queen pheromone on larvae has been found. This series of control, or switch, points probably arose one at a time, with historically newer control points preceding older ones during larval development. Mechanisms that operate early in development limit the power of controls that come later (Michener, 1974). Accordingly, the sequence of control points found in *P. pygmaea*, and presumably other ants, suggests that nutritional regulation of caste was the primitive form of control. Then, the nutritional switch point was preceded by a point regulated by pheromones. Finally, climate became an important component of caste determination when winter dormancy in the last instar was incorporated as a requirement for queen development (Passera, 1969).

The evolution of social control over nutrition may have been particularly important in the evolution of physical castes. The developmental link between nutrition and expression of caste specific characters may represent a weak point during which the fate of individuals can be controlled by other colony members. In eusocial Hymenoptera with morphologically distinct queens and workers, the mechanisms used by queens to control development fall into two types

which may represent two different evolutionary solutions to regulation of offspring development. In the first, queens control larval nutrition, most commonly by controlling the behavior of workers towards brood (eg. honeybees). The effect of queens on larvae is therefore indirect. In the second, pheromones may affect larval development directly, altering the developmental response of larvae to nutrition (eg. Röseler, 1977, Wheeler and Nijhout, 1984). In some ants, queens intervene during embryogenesis rather than larval life, eliminating any effect of larval nutrition in the determination process (Passera and Suzzoni, 1979).

The evolution of multiple worker castes cannot be independent of the developmental mechanisms that guide queen determination. Developmental mechanisms that govern the complexity of the worker caste must be superimposed on whatever system of queen determination already exists. Some systems of queen determination, especially those dependent on regulation of larval nutrition, may constrain the evolution of physical complexity in worker castes (Wheeler, 1986).

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Changes in Ecdysteroid and Juvenile Hormone Hemolymph Titer of Honey Bee Queens During Pupal and Adult Development

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The two primary insect hormones, ecdysterone and juvenile hormone (JH), control embryonic development, growth, moulting and metamorphosis. In most insects, juvenile hormone also regulates ovarian activity. The determination of the hormone hemolymph titers is essential for the understanding of their role in pupal and adult development of honey bee queens.

MATERIALS AND METHODS

Hemolymph of pupal and imaginal stages was collected with glass capillaries after abdominal insertion. Ecdysteroids were measured by radioimmunoassay (Borst and O'Connor, 1974). Juvenile hormone activity was analyzed by *Galleria* bioassay (De Wilde et al., 1968). Juvenile hormone synthesis of the corpora allata was determined by a radiochemical assay in vitro (Pratt and Tobe, 1974).

RESULTS AND DISCUSSION

In the pupal stage, an ecdysteroid peak (1.4×10^{-6} M) occurs shortly after the pupal moult. This peak probably initiates particular developmental events such as apolysis of the pupal and formation of the imaginal cuticle. At a later stage in pupal development, the ecdysteroid titer decreases continuously by two orders of magnitude. Juvenile hormone activity follows an opposite course: After a JH peak in the spinning stage of the last larval instar, the JH titer drops drastically and remains low during the pupal stage (400 *Galleria* units (GU)/ml). Fifteen hours before adult eclosion, the JH titer increases and reaches 5000 GU/ml one day after emergence. The titer changes correspond to changes in corpora allata volume and JH synthesis. The pattern of variation of both hormone titers in honey bee queen pupae coincides with the developmental sequence of hormonal releases as found in other holometabolous insects.

During imaginal life, the hemolymph contains a rather low ecdysteroid concentration ($1 - 3 \times 10^{-8}$ M). In the egg-laying queen, however, the ecdysteroid titer is significantly higher (2.7×10^{-6} M) than the titers found in young queens and adult worker honey bees. In contrast, the JH titer remains high during the imaginal life of a queen and is not modulated in relation to yolk protein synthesis and egg-laying activity as might have been expected from studies on other insects. JH plays a major role in the regulation of age-dependent polyethism in worker honey bees but its function in adult queens still remains unrevealed.

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Genetics of Sex and Caste Determination in Bees

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A revision of hypotheses on sex and caste determination in stingless bees (Meliponinae) is given. Sex presumably is determined by two sets of genes, one acting early in embryonic development, and the other in the prepupal stage. Caste differentiation is drastically dependent on the juvenile hormone titer during relatively late developmental phases. Above a given threshold it is thought to turn on femaleness determining genes. Subsequently the prepupae become queens. Below the threshold, maleness determining genes make female prepupae to develop into workers.

Biochemical Basis for Caste Differentiation in Termites

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Juvenile hormone has a key role in insect polymorphisms including caste differentiation in social insects (Hardie and Lees, 1985). In termites, JH has been implicated in the control of the production of soldiers and replacement reproductives (Watson et al., 1984), and the high JH III titer in the *Macrotermes* queen suggests a role in vitellogenesis. JH analogs, e.g., methoprene, can induce worker to presoldier to soldier molts in both lower and higher termites. However, despite the key role for JH in termite polymorphisms, and a single report of a JH binding protein in *Macrotermes* (Wyss-Huber, 1981), there are no reports of specific JH-binding proteins involved in caste differentiation. We now describe the use of photoaffinity labeling to characterize the first such protein from the hemolymph of the Eastern subterranean termite, *Reticulitermes flavipes*. In addition, we show that both soldiers and nymphs can retard the differentiation of workers to soldiers.

MATERIALS AND METHODS

Reticulitermes flavipes colonies were collected on Long Island, New York, and termites were held at 28° C, 65% RH. Hemolymph from chilled insects was collected in two ways: (1) cutting at the first thoracic segment and centrifuging the posterior section of 10-20 individuals at either 3,000 or 10,000 x g through glass wool in nested microfuge tubes; (2) collection into a capillary from a cut in the thoracic intersegmental membrane, thus avoiding contamination from intestinal material. Induction of (pre)soldiers was performed using 40 ppm (7S)-methoprene (Zoecon Co.). We modified the Howard and Haverty (1979) procedure by using Whatman No. 1 filter paper; this gave faster and more reproducible presoldier induction. The influence of soldiers and nymphs on worker differentiation was assessed as follows. Twelve replicates of twenty workers each were held (a) without methoprene, (b) with methoprene, and (c) with methoprene and the addition of either one soldier, one nymph, one soldier plus one nymph. Six replicates of each situation were used for hemolymph collection by the capillary method.

[³H]-EFDA was prepared by W.-s. Eng (Eng and Prestwich, 1986). Photoaffinity labeling experiments were carried out in the presence of protease and JH esterase inhibitors as described by Prestwich et al. (1985) using 1 μM [³H]-EFDA with irradiation at 4° C for 30 sec with 254 nm Rayonet lamps. Denaturing polyacrylamide gels (SDS-PAGE) at 8% T was used, with PPO-acetic acid enhancement and Xomat XAR-5 film at -80° C for fluorescent autoradiography.

RESULTS

Hemolymph from workers, presoldiers, soldiers, and nymphs from natural colonies, and from workers kept isolated for 30 days was photoaffinity labeled with [^3H]-EFDA in the absence of protecting hormone, in the presence of methoprene, and in the presence of JH III (both micromolar). The proteins were separated on a denaturing gel. Many photoaffinity labeled bands were seen in the unprotected lanes (detected by fluorography) for each caste. Specific JH binding proteins were identified by the absence of a labeled band in the presence of excess JH III. We find that in centrifuged blood, one particular JHBP is present exclusively in the isolated workers; it is absent in presoldiers, soldiers, and natural workers. It is not competeable by methoprene. In addition, an abundant high molecular weight ($>200,000$ daltons) binding protein can be detected in nymphs.

In the absence of methoprene, no presoldier formation was seen in a 14-day period. With methoprene, presoldiers were first observed at day 6, with $22.5 \pm 4.8\%$ differentiation at day 10 and $43.6 \pm 12.7\%$ at day 14. The presence of a single soldier reduced the day 10 value to $5.8 \pm 7.4\%$, a single nymph reduced it to $15.0 \pm 1.8\%$, and the combined soldier plus nymph showed essentially no presoldier formation at day 10. At day 14, over 50% of the workers held with a soldier plus nymph molted into presoldiers, following the molt of the nymph to a nymph-presoldier intercaste.

Further studies of the role of the hemolymph proteins and the chemicals influencing differentiation are in progress. Radiolabeled hydroprone and iodovinylmethoprenol will be used to characterize JHA binding components.

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Reproductive Caste Differentiation and Endocrine Gland Activities in Females of *Odontotermes stercorivorus* (Termitidae, Macrotermitinae)

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The paper deals with the onset of differentiation of the female reproductive caste during the 1st larval instar of East-African *Odontotermes stercorivorus* (Sj.) (Termitidae, Macrotermitinae).

Gonads, corpora allata (CA), and prothoracic glands (PG) of female embryos, 1st and 2nd instar larvae as well as 1st instar nymphs were compared histologically in serial sections. Volume changes of CA and PG were measured. Every instar was subdivided into 6 developmental phases using integumental structure and the No. of epidermal mitoses.

1st instar larvae of *Odontotermes* are morphologically uniform. But already from the 2nd developmental phase of the 1st instar onwards two groups of individuals can be distinguished anatomically. Prospective reproductives are characterized by gonad differentiation and the prominent increase of CA and PG volume changes. Prospective non-reproductives, however, have fully undeveloped gonads and the CA and PG volumes change on a lower level. In case of prospective reproductives the characteristic increase of CA and PG volumes coincides with the onset of gonad differentiation. Time-limited and reversible volume changes detected might indicate producing and secreting activities of CA and PG. - Both developmental lines separate shortly after the beginning of the 1st larval instar. The separation is indicated by the onset of gonad differentiation and the diverging development of the endocrine glands.

Developmental phases 2 and 3 are not only characterized by the maximal CA and PG volume increase in prospective reproductives but also by the greatest No. of prospective non-reproductives having gonadal mitoses. Finally, developmental phases 2 and 3 coincide with the main phases of epidermal mitotic activity during the molt/intermolt cycle. - The developmental phase of the increased mitotic activity might be utilized to shift the volume changes of CA and PG of prospective reproductives to a higher level.

Some facts detected seem to support the idea that female reproductive caste differentiation might be induced by stimulating competent embryos or very young 1st instar larvae and not by a time-limited interruption of their inhibition: 1. Differentiation of female gonads takes place in a minority of 1st instar larvae only. 2. The No. of prospective reproductives and 1st instar nymphs respectively, found in the nests, is very low, i.e. below 3% of larvae of the same age. 3. Prospective reproductives of a distinct area appear over a period of approx. 6 weeks. - Financially supported by DFG.

Regulation of Vitellogenin Synthesis in Honey Bee Queens

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Vitellogenin (VG), the yolk protein precursor, is produced in insects by the fat body of vitellogenic females. It is secreted into the hemolymph and thereafter taken up by pinocytosis into the growing oocytes. In many insect species the transcription of the vitellogenin gene is initiated and controlled by juvenile hormone (JH) which is synthesized in the corpora allata (CA). This hormone also stimulates incorporation of vitellogenin into vitellogenic follicles in most insects (Engelmann, 1979).

In honey bees (*Apis mellifera* L.), the regulation of fertility is as yet unresolved, the data are controverse. In worker honey bees extirpation of the CA prevents vitellogenin synthesis, and JH application restores and stimulates vitellogenin synthesis (Imboden et al., 1976, Rutz et al., 1976), whereas in egg laying queens - capable of laying up to a few thousand eggs per day - allatectomy neither inhibits vitellogenin synthesis nor egg-production and oviposition (Ramamurty and Engels, 1977). In this study initiation and regulation of vitellogenin synthesis in queens were examined using primarily an in vitro approach.

MATERIALS AND METHODS

Honey bee queens were kept in small experimental colonies (Kirchhainer nuclei). Their rates of egg production were noted. In vivo vitellogenin synthesis was analyzed - after ^3H -leucine injection into the hemocoel and one hour of incubation - by indirect immunoprecipitation of newly synthesized, labeled hemolymph proteins with a vitellogenin-specific antiserum. Tracer incorporation into total hemolymph protein was measured by TCA (10%) precipitation of either hemolymph (in vivo) or culture medium (in vitro). Questions concerning regulation of vitellogenin synthesis have been examined by culturing fat bodies in vitro (Kaatz et al., 1985) and adding components such as JH, ecdysterone, hemolymph or head extracts to the medium. VG synthesis of the explanted fat bodies has been analyzed by indirect immunoprecipitation after one hour of incubation in radiolabeled medium (^3H -leucine, 25 $\mu\text{Ci/ml}$).

RESULTS AND DISCUSSION

In the course of queen ontogenesis vitellogenin first appears in the hemolymph of pharate adults, 10 hours before imaginal moult. It is a typical adult hemolymph protein produced by the imaginal fat body together with a number of other imago-specific proteins. Decapitation and allatectomy experiments on pupae, at different stages of development, did not later inhibit the production of vitellogenin. In other words, the initiation of vitellogenin synthesis in the imaginal fat body of honey bee queens is not dependent on JH and factors from the brain. This process might be interpreted as a part of an autogenous protodifferentiation of the imaginal fat body. The conclusion is indirectly supported by the observation that VG also appears in drone honey bee hemolymph (Trenczek and Engels, 1986). These results contradict the JH-dependent initiation of vitellogenin synthesis

in many other insects (Engelmann, 1979). Since VG production in the honey bee queen starts already at an age when ovaries are in a previtellogenic resting stage, it can be concluded that synthesis and follicular sequestration of VG in the honey bee - in contrast to the majority of other insects - obviously are at least partially independent processes.

After the mating flight, the rate of VG synthesis increases approximately three times. At the same time the ovaries grow and start to take up VG. In the egg-laying queen, VG synthesis reaches its highest level and is correlated with the actual rate of egg production. However, it never decreases to zero level: non egg-laying queens still produce VG at a rate that amounts to one third of the maximal rate found in egg-laying queens.

The cultured queen fat bodies react in different modes to factors added to the medium: Juvenile hormone III affects neither rates of total hemolymph protein synthesis nor specific vitellogenin synthesis. Ecdysterone (3×10^{-8} M), known to be involved in the regulation of vitellogenin synthesis in Diptera (Hagedorn, 1985), stimulates protein synthesis in the explanted honey bee fat bodies but not specifically vitellogenin synthesis. Head extracts of egg-laying queens added to the culture medium result in a 2,5-fold increase of VG synthesis by the explanted fat bodies of egg-laying queens. Drone head extracts do not have any effect. Hemolymph (10%) of egg-laying queens added to the culture medium also stimulates VG synthesis. This is not observed in medium containing heat-inactivated or drone hemolymph. Thus a humoral factor(s) other than JH and possibly originating from the head controls and modulates VG synthesis in the honey bee queen in contrast to the interpretation given for worker honey bees by Imboden et al., 1976.

Both phenomena, initiation and regulation of vitellogenin synthesis as non-dependent on JH, might be explained by the fact that the highly eusocial honey bees evolved a potentially permanent mode of oogenesis thus diverging from the original type of cyclic oogenesis found in many insect species (Adams, 1980). Cyclic oogenesis is presumably the result of an intricate control of the CA activity. Permanent egg production, as found in honey bee queens, does not require such a mode of hormonal regulation and enables a more rapid reaction of the queens to changing colonial conditions. However, the hemolymph of egg-laying queens does contain JH (Kaatz, this volume) but its function is as yet unknown. JH might be involved in processes of fat body differentiation such as proliferation of the ER (Della-Cioppa and Engelmann, 1984) or induction of polyploidy in the fat body nuclei as demonstrated in *Locusta* (Nair et al., 1981).

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Ontogenetic Patterns of Vitellogenin Titer and Synthesis in Drone Honey Bees (*Apis mellifera*)

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In most insects species the occurrence of the yolk precursor protein vitellogenin is restricted to females, but there are some exceptions. In honey bees vitellogenin was found in the hemolymph of queens and workers. The question of vitellogenin production in male hymenoptera is of particular interest because of the haplo-diploid sex system. By means of highly sensitive immunotechniques and in tracer experiments, the expression of the vitellogenin gene was analysed in normal haploid as well as in diploid adult drones.

MATERIALS AND METHODS

Drones of known age were taken from *Apis mellifera carnica* colonies. Diploid drones were reared in the laboratory for the first days after hatching and then retransferred onto drone combs. A highly specific anti-vitellogenin-serum was obtained from rabbits using worker vitellogenin as an antigene. Protein separation was done by gradient SDS-PAGE on 5.0-12.5 % gels. Vitellogenin detection by western blots and quantification by rocket immuno electrophoresis was routinely applied. In vivo labelling of newly synthesized hemolymph proteins was effected by injection of ^3H -amino acids and by subsequent fluorographic quantification on slab gels.

RESULTS

Vitellogenin undoubtedly does occur in male honey bees and was detected in the hemolymph of both haploid and diploid drones soon after emergence. The vitellogenin titer in diploid drones was extremely low. In haploid drones the vitellogenin content of the hemolymph was found to increase during the first days of imaginal life. A decrease in titer was observed after day 10. In drones older than 20 days only traces of vitellogenin were detected.

The rate of vitellogenin synthesis in adult haploid drones greatly increased up to day 3 or 4. At this age vitellogenin synthesis amounted to about 10 % of the total hemolymph protein production. Vitellogenin synthesis diminished from day 5 or 6 onwards and was undetectable in most of the drones after day 10.

DISCUSSION

In the honey bee vitellogenin is regularly present in the hemolymph of adult haploid and diploid drones. Therefore, in bees vitellogenin cannot be regarded as a female specific protein. The vitellogenin titer is lower in males than it is in females (workers and especially queens), but the ontogenetic patterns of vitellogenin titer and synthesis are similar to those found in queenright worker bees. Although there is no use for a typical female protein (as a precursor of the yolk protein vitellin) in male bees lacking ovaries, the determined rate of vitellogenin synthesis in normal haploid drones is surprisingly high. It would appear that the expression of an originally female character is insufficiently controlled in male hymenoptera.

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Juvenile Hormone and Worker Behaviour in the Bumblebee *Bombus terrestris*

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In honeybee workers injection of a small amount of juvenile hormone (JH) induces vitellogenin synthesis, whereas a high dose causes a shift from housebee to forager (Rutz et al., 1976) and decreases worker aggressiveness (Breed, 1983). In the closely related bumblebees, however, foragers are characterized by a low JH titre in their haemolymph, whereas injection of JH causes oogenesis (Röseler, 1977). Induction of oogenesis by JH was also observed in halictine bees (Bell, 1973) and in polistine wasps (Röseler et al., 1984), and moreover, in the latter of them, an increase in dominance and/or aggressive behaviour.

MATERIALS AND METHODS

A quantity of 50 µg JH I (which is enough to induce complete oogenesis, taking about 5 days (Röseler, 1977)), dissolved in 4 µl triolein, was injected in the abdomen of freshly emerged workers. In a first experiment the impact of JH on the expression of foraging behaviour, aggression, egg laying and its associated behaviours and of dominance was investigated in two colonies, which got offered honey-water in a flight cage in daylight. Detailed descriptions of behaviours and methods can be found in v. Doorn and Heringa (1986). One fourth of the workers received JH, one fourth only the solvent and the rest remained untreated. Secondly, JH was injected in one out of a group of 4 workers which were kept queenless. The other 3 remained untreated. It was investigated which worker became dominant.

RESULTS

JH treatment did neither affect the proportion of workers which became foragers, nor the age at which they started foraging. The data on aggression did not show a JH effect. The same holds for the proportion of workers which became egglayers or performed behaviours associated with egg laying without actually laying eggs. Dissection of many non-egg-laying workers at the end of the observations showed that their level of ovarian development had become comparable to that of the control workers. Consistent with this, the analysis of the interaction structure in one colony showed that the same proportion of the JH treated workers as of the control workers belonged to the most dominant, the so-called elite workers in the period of worker egg laying. Only elite workers may become egglayers (v. Doorn and Heringa, 1986). Thus no long term effect on ovarian development as well as on dominance behaviour could be observed. Since JH indeed has a short term effect on oogenesis I compared the activity and dominance of JH treated and control workers during the first 5-10 days after injection. No striking differences were found.

To exclude a possible masking influence by social factors it was investigated if a worker could be given an advantage in becoming dominant

in a small queenless group by injection of JH. The JH treated worker became dominant in 11 out of 35 groups (31%), representing a slight, but not significant increase compared to the expected 25%. In these groups a significant correlation existed between rank in the hierarchy and corpora allata (CA) volume at an age of 1-2 days, like in untreated groups, but 1. of the 7 JH treated workers which became dominant only 57% possessed CA with volume rank 1 or 2, against 77% of the dominant workers in the control groups (N=17), 2. JH treated workers had a significantly lower CA rank than would be expected from their dominance status if compared to the workers of the control groups, and 3. on average, JH treated workers had significantly smaller CA than the untreated workers out of their own groups. JH treated workers of 4-5 days of age differed significantly from the workers of control groups by a lower CA rank than expected from their position in the hierarchy, but all the JH treated dominant workers (N=4) had CA rank 1 or 2, whereas the mean CA volume of treated and untreated workers did not differ significantly.

The data show that in queenless groups JH treatment indeed may favour a worker when the hierarchy is established. The injected JH apparently also inhibits the growth of the worker's CA for some time. In intact colonies the effect of JH on dominance seems to be overridden by social factors. Since JH injection did not affect foraging behaviour, it is improbable that a bumblebee worker becomes a forager because of its low JH titre.

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Posture of the Queen Honeybee during Egg Deposition

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When the queen honeybee starts depositing an egg she at first pushes her head into a honeycomb cell for examination. Then she walks over the cell and in a backward movement thrusts her abdomen into it. In this situation her head may be oriented in any direction, looking downwards, upwards or towards the side. In the latter cases she then turns round until her head is oriented downwards at an angle of 0° , 30° or 60° to the vertical. In these positions the queen due to honeycomb geometry finds a symmetrical support for her legs. It is only in this position that she proceeds to stick the egg with its posterior pole to the bottom of the cell where it stands out horizontally. This peculiar behaviour has two consequences.

1) All eggs in a honeycomb display the same dorso-ventral orientation, the concave side of the slightly curved and oblong egg being oriented downward. As a result of this orientation the embryo develops above the yolk, along the convex upper part of the egg and with its belly facing upward. It is only at the beginning of hatching that the larva turns around the longitudinal axis and starts its flexing movements with the ventral side oriented downwards.

2) When the queen honeybee begins depositing on an empty honeycomb, a peculiar pattern of egg distribution emerges. During egg deposition the queen's head is located directly in front of the second but next cell vertically or obliquely below the one being filled. It is this cell into which she pushes her head for inspection in the same movement that pulls her abdomen out of the cell just filled. This results in vertical or oblique "streets" on the honeycomb in which every second cell is filled with an egg. As soon as the queen has reached the lower border of the honeycomb, she will deposit the rest of the current egg batch there, because she is reorienting downwards again whenever she has accepted an empty cell beside or above the one previously filled. This special behaviour may contribute to the fact that the brood is found essentially in the lower parts of the beehive. Other parameters like season, temperature, deposition of pollen and honey, or the age of the honeycomb may provide further cues for establishing the spatial order of the beehive.

I thank the DFG for financial support.

3.3 Genetics and Social Behaviour

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Towards a Sociogenetics of Social Insects

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Social insects, because of their ubiquity, complex behavior, and the large numbers of individuals afforded by colonial life, have attracted the attention of many scientists. Much of this interest has been because of their sociality, but much of it has arisen simply because social insects are abundant, important, and convenient. Many such projects have been both insightful and useful, and I am not arguing for their cessation, but rather for more work on social insects as social insects. Despite all their interest in the evolution of eusociality, and its maintenance, I suggest that much more can be done to develop a sociogenetics of social insects.

This Congress, which has more genetics in it than ever before, may well see a quantum leap in the development of social insect sociogenetics. In this paper, I will consider some examples of the genetic approach to insect sociality, suggest that the importation of molecular biology into such studies will be helpful and not competitive, and lastly hope for a greater unity between different kinds of science as applied to social insects.

KARYOTYPE EVOLUTION

For a long time, karyotype studies on ants had much more to do with their ubiquity and abundance than with their sociality. In fact, they do make a superb group for studies on karyotype evolution, because of the immense diversity of karyotypes that they present. For example, these studies led to the suggestion that chromosome numbers tend to increase during evolution, and not to decrease as commonly supposed. Nationalistically, I will mention that this entire range is shown by Australian genera. Particularly striking is the genus Myrmecia, for example, M. brevinoda has a haploid number of 42 and one of the sibling species included under M. pilosula has one of one (Crosland and Crozier, 1986). We have certainly struck one of the natural limits to chromosome number change!

The connection to sociality was seen by Sherman, who pointed out that groups would be more harmoniously social if their members had much the same level of relatedness to each other throughout. Otherwise altruistic individuals could concentrate on those most like themselves, and group cohesion would suffer. High chromosome numbers decrease the variance in relatedness and low chromosome numbers increase it. Hence, Sherman argued, eusocial groups should tend to have higher chromosome numbers.

Pleasingly, social insects do tend to have higher chromosome numbers than their nearest relatives (despite Myrmecia pilosula!). But the implications of this trend remain unclear, because it is hard, although not impossible, to test whether high chromosome numbers come first and predispose a group towards sociality, or whether sociality fosters increase in chromosome number!

SEX DETERMINATION AND MATING PATTERNS

Studies on social insect chromosomes, although interesting in themselves, are not strongly connected with studies on sociality. Such also appeared to be the case for studies on sex determination (apart from the general consequences of male haploidy for eusocial evolution: Crozier, 1985). Thus, it was nice to know that forms as disparate as Bracon and Apis (but not many parasitoids) have a simple form of sex-determination in which heterozygotes at a certain locus become females and other genotypes become males, but the relationship to social behavior seemed remote until Page noted that (a) diploid drone production imposes a load on colony growth, (b) multiple mating reduces the variance of this load, and (c) under certain conditions of colony growth such reduced variance is favored. This variance-reduction explanation is one of the most plausible (Crozier and Page, 1985) as a cause for multiple mating in some species (in which colony reproduction is near the end of the sigmoidal colony growth curve) and single mating in others (in which reproduction occurs during the exponential increase phase). Various predictions stem from this observation, and should guide further research.

GENETICS OF SOCIAL BEHAVIOR

Although there should be many more of such studies, direct studies on the genetics of social behavior are certainly not lacking. Two such studies are those of Rothenbuhler on honeybee strains behaviorally resistant to foul brood; and, more recently, the ingenious use by Moritz and Hillesheim of the Cape honeybee to estimate heritabilities for behavioral and other traits. The discovery of genuinely high heritabilities for several traits is interesting but also suggests that there has not been much selection on the characteristics involved, which is slightly puzzling.

KIN-RECOGNITION: POSSIBILITIES AND, POSSIBLY, IMPOSSIBILITIES

Kin recognition is certainly quintessentially social, and, to an unknown degree, genetic. Both theory and experiment are important in this rapidly-developing field. The chief questions under study seem to be: the ontogeny of recognition behavior, the source of the "labels", or cues, invoking recognition and discrimination, and the source of the template with which an individual compares another in reaching a decision about the relatedness of the unknown to itself. By "label" (also called "cue"), is meant a characteristic used by other individuals in reaching a decision about classification of the individual under study with respect to degree of relatedness.

The sources of labels and templates are necessarily strongly interrelated, in that they have to be well-matched for the system to function. Much work on the genetic aspects of kin recognition (see Crozier, 1986) has shown that, whatever their origins, labels in social insects and other organisms with advanced social systems are probably most often learned. But the pathways by which the labels to be learned are derived are complex: some will be directly specified by the individual's genetic makeup, some acquired directly from the environment (such as the nest environs) and some acquired via food or symbionts. The genotype will, of course, mediate where a creature lives, what it eats, and which symbionts can live on or in it, so that untangling the relative strengths of these sources in nature is a major task yet to be seriously tackled. The task is not made easier by the fact that, as for heritability, the balance between "genetic" and "environmental" influences will vary with both the genetic composition of a population and

the environment it finds itself in.

As seen by theoreticians, and increasingly demonstrated by experimentalists, kin-recognition systems vary in the sources of the templates and in the kinds of matching employed between template and label. The source could be the animal itself, a subset of its group, or the whole group. The precision of the matching required may also vary from genotypic identity of the unknown to one previously known to be kin, to foreign label rejection (if the unknown has labels not found in the template), to common label acceptance (if the unknown has a label already present in the template). On top of this system one could ask how many correct matches are needed before an individual is accepted as kin. The threshold set for such acceptance will be set not only in regard to the likely error but also to its consequences.

We would expect that individuals would possess more than one template, and react differently to unknowns of the same degree of relatedness depending on circumstances. The reaction with regard to incest avoidance will differ from that with regard to nest defence.

The experimental results so far are characterised as much by diversity as by unity. Thus, Gamboa and co-workers have stressed the importance of exposure to nest material in Polistes, and Lenoir and co-workers find the larva to be the key template learning stage in Cataglyphis, whereas, in contrast, several other groups find that adulthood is the key learning stage and that adults provide the templates. Within this latter group, Carlin and Hölldobler find that queen-derived labels are usually the most important in various Camponotus species, whereas Mintzer for Pseudomyrmex and Michener's coworkers for Lasioglossum zephyrum found that worker-derived cues predominate.

Some of these differences in results may stem from differences in the questions asked, but recent theoretical study of kin-recognition systems in marine invertebrates (Crozier, 1986) indicates that genetic polymorphism for the labels may not be stable for recognition associated with altruism. The marine invertebrates have self-matching and either common-label acceptance or foreign-label rejection, depending on species. The recognition systems of social insects are more complex, involving both male-haploidy (for many) and learning, so that generalization of these results to them is still to come. Preliminary results indicate that, for weak selection and queen-derived labels, the marine invertebrate results hold for social Hymenoptera. The tentative conclusion is that there will not be glands just "for" colony odor: any such glands must serve at least one other function for which diversifying selection is stronger than that imposed by kin recognition. A strong candidate for this role is the immune system.

CASTE AND LEVELS OF SELECTION

It is hard to think of social insects without thinking of caste. One approach has been to search for underlying genetic diversity influencing caste diversity. Kerr and Buschinger have reported such cases, but seems unlikely that such polymorphisms will be found to be a general phenomenon, and some critical tests remain to be done for suggestions linking heterozygosity and caste determination.

Genetic (and developmental) questions that should be asked about social insects include (a) how easily can allometry curves be changed, and (b) how much variation is there in natural populations for the potential to become a reproductive? These topics merge with kin-recognition, in that Oster and Wilson suggest that caste evolution may be dominated by intra-colony selection: where workers have ovaries, reproductive competition between them (involving recognition) prevents the development of much

specialization. The available data give at least the overall impression of agreement, but the question remains: which came first?

Such considerations lead naturally to a reminder of the multi-level nature of social insect populations. Colony-level selection, the theory for which is now being considered in depth by Robin Owen, must be a level of selection of considerable importance in social insects, although it forms just one level in an extensive hierarchy (population, confederation, colony, nest, matriline, patriline, individual). Such studies should cease being chiefly the preserve of theoreticians and be taken up vigorously by students of natural populations (sex allocation studies have been an important harbinger of this general approach).

ADDING MOLECULAR BIOLOGY...

Many of the studies advocated above are difficult because suitable genetic variants are lacking with which to dissect the basic architecture of our subjects. Isozyme variants are useful markers for many studies of natural populations, but they are scarcer than we would like and have no discernible effects on caste development, social behavior etc. At least with honeybees, it is now technically possible to import molecular-biological techniques and make specific mutants. The P-elements of *Drosophila* are transposons that cause mutations by inserting themselves at various genomic locations. When a desirable new mutation is detected using this phenomenon, the specific site it is at can be located using molecular probes, and the locus then be cloned, isolated, and characterised. Perhaps now we could find those hygienic loci!

Naturally, molecular techniques also provide a bonanza of characters for phylogenetic analysis, and various vexing evolutionary questions may finally yield to this attack. Such studies can also be used within populations, e.g., the fact that mitochondrial DNA is maternally inherited could be used to trace the migrations of females separately from that of males.

BRINGING TOGETHER TWO KINDS OF SCIENCE

Scientists can be usefully dichotomised in various ways. One such division, for evolutionary biology, is between those who study the forces of selection acting on populations, and those who study the machinery which responds to selection. Communication between these groups has tended to be restricted. This situation should change: the two approaches are complementary and interdependent. A new golden age for social insect studies will emerge when this change arrives.

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SYMPOSIUM

Sociogenetics in Insects

Organizers: Ross H. Crozier, Robin F.A. Moritz,
and Pekka Pamilo

Introduction

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THE IDEA

The analysis of genetical mechanisms in socially structured populations is one of the major aims in sociogenetics, the synthesis of the disciplines sociobiology and genetics. Classically, the genetical study focusses on gene action or interaction in individuals and the analysis of gene expression in individual phenotypes. From sociobiological studies we know that in social populations the picture is more complex. Models used for individual genetic analysis often fail to explain group phenomena sufficiently. Interactions among individuals assembled in social groups may affect gene expression on the intra individual, individual, group, and colony level. The genetic impact on such interactions may strongly affect individual, group and colony phenotypes which are exposed to artificial and natural selection.

For the study of the effects of sociality on genetics and vice versa, social insects provide the most intriguing test organisms. They can be found in a tremendous variety of levels of social organization, ranging from the solitary insect to highly eusocial colony structures. Furthermore social insects are abundant and easily accessible in field as well as in laboratory studies. Also because of the small size of social insects large colonies can be kept under low cost laboratory conditions; social vertebrates usually present much greater problems in maintenance and experimental set up.

QUESTIONS AND ANSWERS

One major question in sociogenetics results from the presence of altruistic behavior in social populations. Why do particular members of a society reduce their own individual fitness in favor of others?

One answer to this question is given in kin selection theory predicting the presence of kin recognition mechanisms. Kin recognition mainly refers to the problem of interactions at the individual level and definitely is one of the central features for kin selection in social insects. Though no paper was specifically dedicated to this topic, the question of kin selection was often raised. Once again it could be experimentally shown that semen clumping in gynes' spermathecae, a rescue hypothesis for kin selection theory in polyandrous social species, cannot be found in social insects. The semen from multiple males repeatedly have been shown to fertilize in constant proportions in genetic marker studies.

Another major selective force in social insects seems to be selection at the group or colony level. What is the position of the group and the colony in selective processes in social populations?

In several presentations, the importance of the group was discussed as a crucial factor for selection in social insects. The arguments supporting group selection theory ranged from theoretical

models, qualitative behavioral observations, to the quantitative analysis of group behavior. The results reported by E. Hillesheim, showing that individual fitness may run counter to group efficiency, provide a plausible answer to the question by Crozier (see plenary address) as to why the traits closely connected to individual fitness analyzed by Moritz and Hillesheim (1985) have high genetic variances. It became clear that selection and population models which invoke individual, group, and colony level selection are most useful to analyze genetic mechanisms in social insects.

An additional factor of selective importance is the sex investment ratio in social insects. Particularly for the male haploid social Hymenoptera, female biased sex ratios have been predicted. Does this hypothesis hold as a general rule for social insects?

For the first time a theoretical framework simultaneously invoking brood group size, local mate competition, and local resource competition was presented. Several field studies revealed the complexity of sex allocation in social insects, in which sample and estimation problems may bias estimates of investment ratios. The work in spiders showed, once again, that biased sex ratios do not depend on male haploidy but also appear in diploid populations.

Interactions among colonies in the population may have effects on the population structure. How does sociality affect the genetic variability within the population?

Population parameters were tested to study interactions at the intercolony level. Termites and ants with polygynous and monogynous forms proved particularly useful in such studies. Recognition mechanisms among colony members seem to become increasingly important for the understanding of the dynamics of colony interactions.

FINAL IMPRESSION

Though the central questions in sociogenetics have been recognized, we are just at the beginning of a detailed understanding of the genetic principles in social populations. Important problems, not spelled out in any of the presented papers, but derived from several, remain to be solved. Intercolonial and interpopulational variation has to be considered as a serious point. Environmental variation, in particular in field studies, often seems not to be adequately considered. For example one should not assume that colonies of the same size even under equal environmental conditions in a given population necessarily have the same investment sex ratio. Also over time investment ratios may change due to environmental variations. Thus only large scale repeated measurements will give reliable estimates from field studies.

With the presentations of various authors in this symposium, we tried to give a brief insight into experiments, theories, and hypothesis resulting from sociogenetic work; not only in social insects but also in social spiders. One point of current interest, kin recognition mechanisms in social insects, is left out here, since it is separately discussed in detail elsewhere in this volume.

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Selection on Group Nesting in Foundresses of *Polistes annularis*

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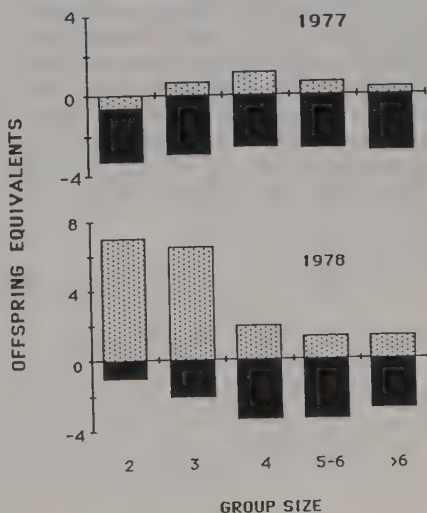
Group nest founding in *Polistes* wasps has been a favorite system for studying the evolution of altruistic behavior because some females who seem to be capable of nesting alone give up most of their reproduction in order to aid queens (e.g. Noonan, 1981). Here we report on an analysis of selection on such altruism in *P. annularis*, studied on a cliff face near Austin, Texas.

Nest watches totalling 212 hours showed that the average subordinate foundress laid only 8.8% as many eggs as her queen, and this figure did not differ with group size. We used this figure to allocate the total number of reproductive females produced (on over 100 nests in two years) among the queen and her subordinates. The resulting values for individual fitness were used in the two analyses described below.

Figure 1 summarizes the results of an inclusive fitness analysis (for details see Queller and Strassmann, 1987). Inclusive fitness analyses can only be performed for binary choices, so we have performed five separate comparisons for each year, each comparing the payoff of joining a nest of size x as a subordinate versus nesting alone. Relatedness of foundresses was estimated by a recursion pedigree method at about 0.5. Subordinates were more successful than single nesters only on nests of size 2 and 3 in 1978. This means that 100% of subordinates in 1977 and 86% in 1978 had a lower expected inclusive fitness than females nesting alone. This conclusion would still hold if relatedness were actually 0.75, the maximum possible for outbred full sisters.

The results of this analysis can be accounted for by one of two possibilities. First, joining and nesting alone may actually be equally profitable choices, but something has been left out of the model. For example, the wasps who joined as subordinates may be in worse physical condition than single nesters, a possibility explored in Queller and Strassmann (1987). Here, we will further investigate the second possibility: that there actually is selection operating against joining as a subordinate. The inclusive fitness analysis does not give a quantitative estimate of the amount of selection against joining; nor does it give a predicted response to selection. One problem is that a set of analyses of five separate binary choices does not generate a single number for the strength of selection against joining.

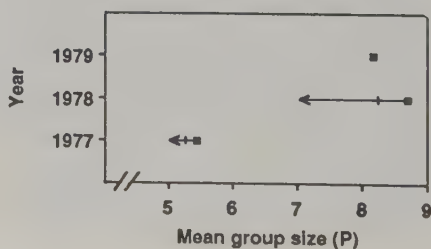
Fig.1. Inclusive fitness of subordinate foundresses in different group sizes. Black bars show the average decrease in output of their progeny compared with singly nesting foundresses. Stippled bars show the average increase a subordinate causes in her queen's progeny, devalued by their relatedness, estimated at 0.5. The sum of the two bars is the inclusive fitness effect of joining versus nesting alone.



A single number can be provided by a quantitative genetic model of selection on the trait of individual choice of group size, designated by P . We have previously explored one such model (Queller and Strassmann, 1987) with the response to selection being predicted by the familiar quantitative genetic equation, $R = Sh^2$, where h^2 is the heritability of P and S is the selection differential (the difference between the selected and unselected means of P). In this simple model each individual—single, subordinate and queen—was scored for P in the calculation of the selection differential. But this ignored an important feature that is included in the inclusive fitness model. There we made the reasonable assumption that group size is determined by the choice between joining a group as a subordinate and nesting alone. Queens were assumed not to affect group size; they would accept any female willing to join as a subordinate. Under this assumption, queens should be regarded as unscored for P , since they have not demonstrated what size group they would have joined if they had not been a queen.

Therefore, it seems that queens should not be included in the calculation of the selection differential. But to exclude them would also be incorrect, for queens do carry and transmit genes for joining, even if they do not express them. One solution, to be derived in detail elsewhere, is to use information from relatives to predict what group size queens would have joined if they had expressed their genes for this trait. Figure 2 shows the predicted selection responses calculated as $R = F_s S_s h^2 + F_q S_q h^2$. Here F_s and F_q are the fractions of the population's total reproduction due to (1) subordinates and singles and (2) queens. S_s and S_q are selection differential calculated separately for each of these groups, with the latter using not the deviation of the queens from the population mean, but the deviation of their subordinates times r . Since h^2 takes a maximum value of 1, the arrows in Fig. 2 give a predicted range for the mean value of P the following year. The prediction for 1978-9 is accurate, but the range predicted in 1977 for 1978 is far too low. Clearly, there must be something that we failed to account for in the model. Perhaps it is only an environmental difference between years that affected group size. But we should also consider the possibility that the kinds of choices made during the joining period are more complicated than we have supposed. Limited nests sites and joining decisions based on individual abilities and past histories are important areas for further investigation.

Fig. 2. Selection on group size. Black squares are mean foundress group sizes for three years. The arrows represent the predicted range for the mean group size of the succeeding year. The part of the arrow to the left of the hatch mark represents selection due to queens, $F_q S_q$, and the part to the right is the selection on subordinates and singles foundresses, $F_s S_s$.



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Genetics of Social Behaviour in Honey Bees (*Apis mellifera* L.)

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Cooperative behavior and intense regulative interactions between members of a social group are basic characteristics of highly eusocial insects. Often colonies of social insects are addressed as superorganisms, since polyethism and the caste system result in many operational similarities to true biological organisms. The fundamental genetical difference to the individual organism is its "intra-individual" genotypic variation. Consequently this results in within-colony selection among individuals. Thus, for understanding selective processes in populations of social insects we will have to consider both individual and group or colony level selection.

Classical quantitative genetic analysis refers to genetic variation among characters of individual organisms only. In social groups, however, we often find behavior which is expressed in groups but not in isolated group members. Individuals may behave differently when removed from their social context. For example colonies of honey bees (*Apis mellifera* L.) operate like homeothermic organisms, whereas each individual worker has to be considered as poikilothermic organism. In this study the alarm reaction is used to quantify effects of worker interactions on the group phenotype in honey bees.

MATERIALS AND METHODS

Honey bees with highly variable alarm behavior had been available from a diallel breeding project for the analysis of defensive behavior (for details see Moritz et al. 1986). Capped worker brood of a low line (L), a high line (H) and two hybrid colonies (LxH and HxL) was allowed to emerge in an incubator (35°C, 60% RH). Freshly emerged workers were placed in groups of 40 into screened containers and fed with pollen and honey ad libitum. Various compositions of these groups were tested. Pure L, H, LxH and HxL groups, and mixed groups of L and H bees (L+H) were established. Also, freshly emerged worker bees from two unrelated colonies with similar reactivity to IPA, were combined at different ratios. After 4 days the groups were tested in a metabolic bioassay described previously (Moritz et al. 1985). The test groups were exposed to a 5 second flush of isopentyl acetate, a major compound of the alarm pheromone, and the reaction of the test bees was quantified by measuring the short term increase of O₂-consumption or CO₂ production.

RESULTS

Fig.1a shows the results of the first set of experiments in which workers with highly different phenotypes were combined (L+H). There is no significant deviation from linearity in this data set, implying that mainly additive interactions between the group members affect the group phenotype. The average reaction of genetic hybrid groups (HxL or LxH) did not significantly differ from the in vitro mixed groups of 20 L

workers with 20 H workers. Fig. 1b shows the reactions of the combined groups of workers from colonies which have a similar low reactivity in the present bioassay. There is a significant deviation from linearity and mixed groups apparently reacted stronger than pure groups.

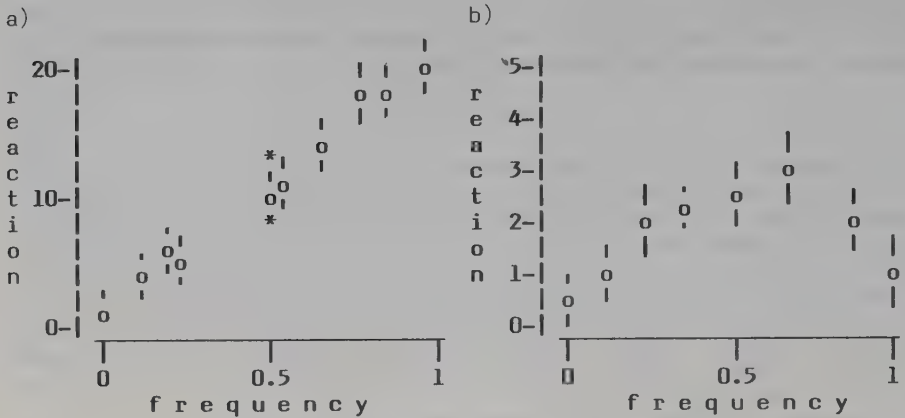


Fig. 1. Reaction of mixed test groups to a 5 second exposure of IPA. X-axis: frequency of highly reactive workers in the group; Y-axis: O_2 -consumption (a) or CO_2 -production (b) in μl /bee/min. a) Test groups are combinations of 40 workers from preselected H and L colonies. \circ = mean \pm SE of 5 test groups; * = mean of 40 test groups from genetical hybrid colonies (HxL and LxH respectively). b) Workers from colonies with similar reactivity were combined in various ratios.

DISCUSSION

Group behavior is determined by both additive and non-additive interactions of the group members. In the case of strong phenotypic differences, the additive variance overrode the nonlinear interactions. The non-additive component only appeared when workers with similar phenotypes were combined. The non-linear interactions found in this study are similar to overdominance phenomena found for individuals. Reactions of the mixed genotype groups were stronger than those of the strongest reacting pure group. Since overdominance is thought to be one of the key factors for heterosis and hybrid vigor, genotypic within-group variability might similarly affect group vigor. In this light the multiple mating of honeybee queens also may be a result of selection for genetic heterogeneity within the colony.

ACKNOWLEDGEMENT:

I wish to thank E.E. Southwick for many stimulating discussions and the Alexander von Humboldt-Stiftung for generously funding this study.

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Experimental Inbreeding, Alate Sex Ratios and Incompatibility between Colonies of the Acacia Ant *Pseudomyrmex ferruginea*

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The ant *P. ferruginea* is an obligate inhabitant of the swollen thorn acacias of lowland tropical Mexico and Central America. In 1979, colony founding females were collected at five localities in Tamaulipas and San Luis Potosi, Mexico. These ants were installed on a clonal population of *Acacia hindsii* propagated from rootstock and stem cuttings, and founded a greenhouse ant population. In the years since then, I have reared two descendant colony generations. The mature colonies rear alate sexual forms throughout the year, and these alates will mate in the greenhouse in flight cages and plastic vials. I have conducted two generations of inbreeding to test very simple genetic models for odor cues utilized to discriminate non-nestmates, and sex determination.

EXPERIMENTAL INBREEDING AND INTERCOLONY INCOMPATIBILITY

In the field, aggression is noted between colonies of *P. ferruginea*, and this incompatibility between colonies does not disappear when the ants are reared on a clonal acacia population. My results indicate that the ants are responsible for the odor cues facilitating discrimination of non-nestmates. In 1979, Crozier and Dix proposed a series of simple gene models for generating the odor cues. In particular, they distinguished between single locus, multiple alternative allele models and polygenic models with two alternative alleles at each locus. They suggested that an inbreeding program might yield illuminating results, because homozygosity would be achieved much more quickly if only one locus was involved in generating odor cues. Homozygosity within an inbred lineage might lead to high levels of compatibility within such lineages. In contrast, homozygosity would be achieved very slowly by inbreeding if many independent loci are involved. Our results show that incompatibility is reduced very slightly within inbred lineages, supporting a polygenic model. The rejection frequency dropped from 77.7% (N=900) for parental colonies to 68% (N=120), 62.5% (N=200), and 55% (N=20) for three inbred F1 lineages.

However, the age relationship of two colonies used in non-nestmate introductions may be a more powerful predictor of intercolony compatibility than kinship. The highest rejection levels were observed when workers from sexually immature colonies were introduced into mature colonies, and kinship had no effect on these results. The rejection frequency dropped when the same pairing of colonies was repeated after the younger colony had reached maturity.

Even after two rounds of inbreeding, the ants can still distinguish nestmates from non-nestmate kin, which are usually

rejected. In the field, there is no evidence for population viscosity in P. ferruginea. The alates are agile in flight and disperse widely in search of mating swarms and unoccupied host plants. Local assemblages of closely related kin-colonies are unlikely to occur under these conditions, and as a result selection for the discrimination of non-nestmate kin is probably quite weak.

EXPERIMENTAL INBREEDING AND ALATE SEX RATIOS

Acacia ant colonies usually reach maturity and begin producing alates in their second year. Once production of sexuals begins, it continues without interruption. Females greatly outnumber males in the sexual broods of queenright colonies, especially in the first year of alate production. The proportion of males increases slightly between the first and second year of alate production, but females still outnumber males in most cases. Each of seven colonies followed for seven years showed an overall female-biased numerical ratio. A few transient periods of male-biased sexual production lasting 3-4 months were noted in some of these colonies. Workers in queenless colonies produce viable eggs and the proportion of male alates increases significantly as these colonies slowly expire over a 12-18 month period.

Patterns of sexual production are similar in parental and inbred F1 colonies. No significant change in overall alate sex ratios was noted. One simple model (Whiting-Mackensen) for sex determination posits that hemizygous or homozygous genotypes at a series of sex-determination loci result in males. Inbreeding should lead to the production of diploid males under this model. Unless these diploid male genotypes are inviable (i.e., due to developmental lethality or removal by attending nurses), the proportion of males should increase in inbred colonies. My results suggest that this model may not apply to P. ferruginea unless colonies are efficiently removing larval male diploids, or the number of independent sex-determining loci is very large.

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Sociogenetics in *Reticulitermes* Termites - The Nest: a Family, a Tribe or a Population?

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Termite nests are generally considered to be founded by a couple of sexuals who meet after swarming. A nest, at the beginning of its history, is a family. But reproduction can be carried out in this genus by both sexual alates and neotenics (1,8). In Europe, dispersal is achieved both by swarming and budding (workers build long galleries in the soil between pieces of woods and part of the society can be separated from its original nest site by a considerable distance. In these peripheral part of the nest, neotenics assume reproduction and in theory increase inbreeding). The paramount problem of sociogenetics in *Reticulitermes* species is to determine if

nests are families or tribes in closed societies or populations in open societies where members could accept alien homospecific individuals, including neotenics. We studied this problem in European termites (Fig.1) using two complementary techniques: ethological analysis to determine the open or closed nature of societies and genetic analysis using enzymatic polymorphism to characterize the number of reproducers (4)

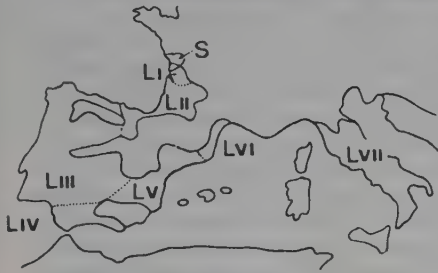


Fig. 1.—Geographical distribution of *Reticulitermes* species:
S: *R. santonensis*, LI, LII: *R. (lucifugus)*
grassei, LIII: *R. (l.) grassei* X *R. (l.)*
banyulensis, LIV LV LVI : *R. (l.) banyulensis*
LVII : *R. (l.) lucifugus* .

MATERIALS AND METHODS

To determine the level of agonism between nests in the same population we used mixed groups of workers meeting in a neutral area (2). The agonism level was measured with the index $Ag=2.5 (M+m/2)$ where M is the mean number of dead workers and m the mean number of injured workers (Fig. 2) (3,7). The study of enzymatic polymorphism was shown to be a valuable approach to the understanding of termite population genetics (4). Allelic frequencies of some polymorphic genes were directly calculated in each colony using phenotype data given by an electrophoretic

Species and areas	<u>santonensis</u>			<u>lucifugus</u> complex			VI	VII
	S	I	II	III	IV	V		
Colony genetics								
Family	43%	82%	20%	77%	100%	60%	50%	50%
Population	0%	9%	60%	5%	0%	20%	25%	20%
Tribe	57%	9%	20%	18%	0%	20%	25%	30%

Table 1 — Colony genetics in each area

migration. Three cases were considered: 1) The allelic frequency discovered approximated to the theoretical Mendelian frequency: we can state that the colony is a family (Table 1) composed by a pair of alates or neotenics. 2) The allelic frequency was random and the theoretical phenotype calculated with the Hardy-Weinberg law approximated the experimental one: we can consider a colony as a population in itself. 3) If the Hardy-Weinberg law is not confirmed by the statistical test (extra homozygotes or heterozygotes) the colony is a closed and inbred tribe or an open tribe.

RESULTS

Species and areas	<u>santonensis</u>	<u>lucifugus</u> complex					
	S	I/II	III	IV	V	VI	VII
Mean Ag \pm SE between nests	2.2 \pm 1.7	14.9 \pm 9.1	71.4 \pm 17	80.2 \pm 7.3	84.1 \pm 5.2	31.9 \pm 5.8	15.8 \pm 7.7
Percent of open popul. in summer	100	92	45	0	0	55	100

Table 2-- aggression between nests and open populations

Combined ethological and genetical analysis indicated that: 1) In damp areas (S,LI,II,northernLIII,LVII) containing large wet pine forests and abundant decaying wood, societies are always open in *R. santonensis* or during summer in *R. lucifugus* complex: 60% are populations at LII. 2) In dry areas (LIII,LIV,IV) colonies are closed. At LIII 77% of colonies are composed of a family, 100% at LIV and 60% at LV. But the ethological test showed that the closed nature of each nest is not due to a mechanical problem (impossibility of digging galleries) but to behavioral obstacles. 3) Nests are composed of tribe for 57% in *R. santonensis*, 20% in LII, LIII, 20% in LVI and 30% in LVII. These tribes are open or closed and appeared during expansion of the nest after sexual alate dead or separation from the original nest. 4) At the edge of the distribution (LVI/S) inbreeding is higher than within the area. Reproduction is carried out by neotenics. Here the climate prevents colony extension and favours inbred tribe formation. A complementary chemical analysis indicates that cuticular hydrocarbons are responsible for colonial identity. Any normal contact is interrupted between two workers from closed societies when contact chemoreceptors perceive an unfamiliar mixture of compounds (5,6).

Fig. 2.-- Intrapopulation agonism in the *R. lucifugus* complex: oblique hatching covers the regions where societies are open in summer (Ag(25), white areas are inhabited by closed societies (Ag(25)).

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Effects of Eusociality on Population Genetics

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Population genetics plays a central role in the study of evolution, and because the evolution of social insects is of considerable interest it is important to understand how eusociality affects population genetic processes.

Eusocial insects exist in colonies within which there is a reproductive division of labour. A colony may have a complex social structure. In the social Hymenoptera it may be headed by one or more queens (polygyny), which may or may not be multiply-mated and it may physically occupy one or several nests (polydomy). Termites differ in that the colony is headed by a royal couple (king and queen) with one or more supplementary queens taking over as the fecundity of the primary queen declines (Wilson, 1971). Termites have a diploid genetic system whereas all Hymenoptera are haplodiploid (equivalent to X-linked inheritance). The interaction between eusociality and haplodiploidy will be discussed here.

Population genetics - the study of how Mendel's laws apply to populations (Hartl, 1980) - encompasses both deterministic (e.g. selective) and probabilistic (e.g. random genetic drift) processes.

The social structure directly affects processes influencing the statistical distribution of alleles in a population. For instance polygyny and multiple-mating, (a) reduce the average relatedness, \bar{r} , of workers. Specifically $\bar{r} = [(1/4) + (1/2n)]/N$, assuming N unrelated queens, each contributing equally to the worker-force, are each inseminated by n males with their sperm used equally; (b) increase effective population size. Polydomy (e.g. in *Formica* ants) affects the spatial distribution of allele frequencies and the microdifferentiation of populations (Pamilo, 1983).

Selection can act directly on the queens and males (individual selection) or at the level of the colony through the workers. It is assumed that the survival and/or reproductive success of the colony depends on the genotypes of the workers. The outcome of colony-level selection at a single locus with two alleles differs from that due to individual selection because up to three, instead of one, polymorphic equilibria (2 stable, 1 unstable or *visa versa*) can occur. Social structure is also important as multiple-mating increases the number of equilibria possible (Owen, 1986).

The production of males by workers of the Hymenoptera is a consequence both of haplodiploidy and eusociality. Worker-produced males affect the outcome of individual and colony-level selection by altering equilibrium allele frequencies; effective population size is also reduced (Crozier, 1977).

Deterministic and stochastic processes can interact through the social structure to influence each other. Consider sex-determination, which in most Hymenoptera is probably controlled by one or more loci, with individuals heterozygous at least one of

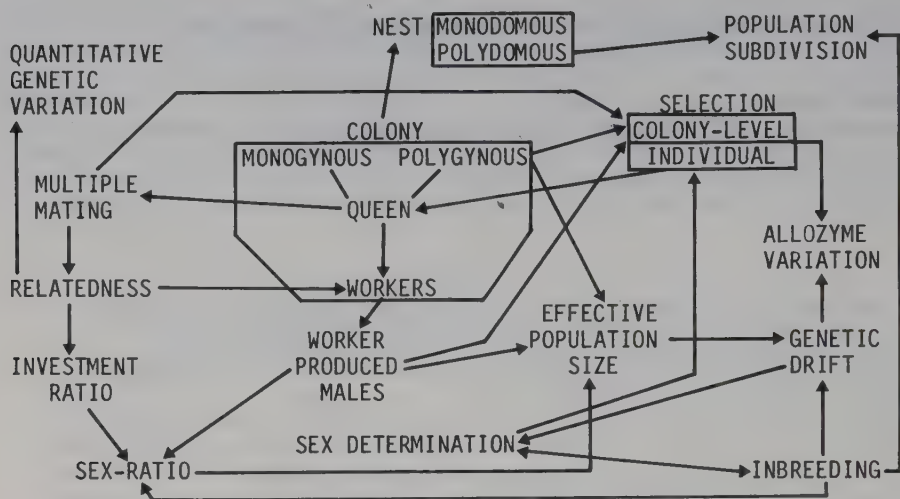


Fig. 1. Some potential and actual interactions between population genetic processes and eusociality.

these developing into females. In some species (e.g. honeybees) selection may favour multiple-mating by queens as this reduces the average proportion of diploid males in the brood, thus increasing the individual fitness of queens (Page, 1980). However the \bar{r} of the workers will be reduced altering the optimum investment and numerical sex-ratios. Multiple-mating and a shift in the sex-ratio will alter effective population size feeding back on the sex-determination loci, because in a finite population sex alleles are maintained by a balance between mutation, drift and selection.

Fig. 1 shows some examples of potential or actual interactions between different population genetic processes and eusociality, the influence of haplodiploidy is pervasive and not explicitly shown. Clearly the effects of eusociality on population genetics are widespread and complex.

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Testing Sex Ratio Theory within Social Insects

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In their pioneering paper of 1976, Trivers and Hare applied sex ratio theory to test alternative hypotheses about evolution of sociality. Many factors governing optimal sex ratios in social insects have been investigated in detail (see Charnov, 1982 for a general review, Pamilo, 1982 for simulations of multilocus/multiallel control, Novacs, 1986 for a recent examination of empirical data). In the present work, effect of the simultaneous influence of local mate competition, finite brood size, male/female dimorphism and differential male/female dispersal on sex ratios is explored.

The Model

An infinite population with non-overlapping generations is assumed. On each of the single foundress nests, in addition to the worker brood, N reproductive offspring are produced, a fraction m being males (probability of an unfertilized egg being laid is m). Dimorphism is modelled by w such that one female is equivalent to w males. A fraction d_M of the males and d_F of females disperse (probability of dispersal is d_M and d_F respectively) to join the mating pool while sibmating takes place amongst those remaining at the nest. Singly inseminated females from the nests and the pool give rise to the next generation.

A single locus is assumed to control the sex ratio, the two alleles acting additively. When a pure population of one allele is invaded by a small proportion of another allele coding for a different sex ratio, the dynamics of the allelic frequency change is expressed by a system of five linear difference equations. The elements of the corresponding matrix are functions of N , d_M , d_F , w and the sex ratios specified by the two alleles. If the dominant eigenvalue of the matrix is greater than 1, invasion is possible. Optimal (evolutionarily stable) sex ratio is the one which cannot be invaded by any alternative sex ratio (for details, see Charnov 1982, Joshi and Gadagkar 1985, Joshi 1986).

Results

Under the joint influence of the two stochastic processes (dispersal and sex determination), some of the females remain unmated on the nest and die without reproduction. The mean number of fertile females emerging

per nest is

$$N (1 - m) [1 - (1 - d_F) (1 - m + m.d_M^w)^{N-1}]$$

When the gyne controls the sex investment ratio , the optimal sex ratio \hat{m} increases monotonically with d_F , reaching 1/2 at $d_F = 1$. The value of \hat{m} decreases (female bias increases) with increasing N , increasing w and increasing d_M . For moderately high values of d_F and for $N > 50$, infinite brood approximation is quite satisfactory.

When the workers control the sex ratio , dependence of \hat{m} on d_F is complicated. For high values of N and w and low values of d_M , \hat{m} increases with d_F to 1/4 as expected. However , for a different set of values , it initially increases with d_F and then decreases. For the parameters ($N=75$, $w=1.0$ and $d_M=0.90$), \hat{m} monotonically decreases with increasing d_F . This unexpected result (increased outbreeding leading to increased female bias) is traced to the complex interplay between genetic relatedness , stochasticity and local mate competition.

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SYMPOSIUM

Genetics of Social Insects

(contributed papers; general introduction see page 331)

**Organizers: Ross H. Crozier, Robin F.A. Moritz,
and Pekka Pamilo**

Genetic Variation in Solitary and Cooperative Spiders of the Genus *Anelosimus* (Araneae: Theridiidae)

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The spider genus *Anelosimus* includes several species which live in cooperative social groups as well as solitary species. In this study genetic variability in two of the cooperative species, *Anelosimus eximius* and *A. domingo*, and a solitary species, *A. studiosus*, are compared.

Anelosimus eximius and *A. domingo* are found in rainforest habitat in South America. *A. studiosus* occurs in a variety of habitats from northern South America to the southeastern United States (Levi 1963).

Colonies of *A. eximius* may contain several hundred or thousand individuals. Colony members cooperate in prey capture and web maintenance; share large prey items; and may guard egg-sacs and regurgitate food for young which are not their own (Christenson 1984). New colonies are founded by mated females which disperse out of their home nests; they may be joined by other dispersing females. Most young appear to mature and reproduce in their home nest. Less is known about the biology of *A. domingo*, but its social system appears to be similar. *A. studiosus* is usually solitary; immatures remain with the mother for several instars, sometimes until they reach adulthood, before dispersing (Brach 1977).

Samples of *A. eximius* were collected from colonies in Panama, Suriname, Ecuador, & Trinidad. Samples of *A. domingo* were collected from Suriname. *A. studiosus* was collected from three localities in the southeastern United States the Archbold Biological Station, Lake Placid Fla. (Highlands County); Radnor Lake, TN. (Davidson County); and Withlacoochee State Forest, Fla. (Hernando County). These samples were screened for genetic polymorphisms in 46 enzymes using starch gel protein electrophoresis. Thirty-seven of these enzymes gave scorable results. Methods and listings of the enzymes screened are given in Smith (in press). For the cooperative species an initial screen examined at least one individual from every colony sampled to detect polymorphic enzymes. Subsequently a minimum of 7 (1984), 10 (1986) or 5 (Trinidad 1985) individuals from each colony were surveyed for their genotypes for each polymorphic locus. Eighty individuals of *A. studiosus* were screened for polymorphisms in the 37 scorable enzymes.

The cooperative species *A. eximius* is highly monomorphic; of 37 scorable enzymes, 7 were polymorphic. Three of these polymorphic enzymes, acid phosphatase (ACP), alcohol dehydrogenase (AHD) and methylumbelliferyl phosphatase (MUP), showed fixed differences between samples from Suriname, Trinidad and Ecuador east of the Andes on one hand, and samples from Panama and Ecuador west of the Andes on the other. The other polymorphic enzymes, an esterase (EST), a super oxide dismutase (SOD), phosphoglucosutase (PMG) and nucleoside phosphorylase (NP), vary within populations; EST, NP, and PGM each have two detectable allozymes, SOD has 3. No more than three loci (8% of the enzymes examined) were found to vary within any one local population.

Members of each colony appear to be genetically very similar, but there is often genetic differentiation among colonies in a local population. A colony from relatively undisturbed rainforest habitat generally consists of individuals which are identical homozygotes at all loci examined: of 28 colonies from undisturbed rainforest sites in Suriname which were examined, only one contained heterozygotes (for PGM; all three expected genotypes were present in Hardy-Weinberg proportions). On the other hand, of 12 colonies collected along a roadside in Suriname, half contained more than one allele at one of the polymorphic loci.

Only three colonies of A. domingo were found. Ten individuals from each colony were screened and results agree with findings for A. eximius: of 37 scorable enzymes only 3 were polymorphic and members of the same colony were identically homozygous at all loci.

The picture is quite different in the solitary A. studiosus. Of the 37 enzymes examined two gave no results, 16 (50%) were polymorphic, an additional five (14%) appeared polymorphic but resolution was too poor to score, and only 13 loci (37%) were monomorphic. The number of allozymes per polymorphic locus ranged from 2 to 5.

Possible avenues for gene flow among cooperative Anelosimus colonies are foundation of new colonies by unrelated females; migration of adult males to new colonies for mating; and migration of females or immatures among established colonies. The fact that adjacent colonies in a local population may be fixed for different alleles at polymorphic loci implies that adult males or other age-sex classes do not routinely migrate among established colonies; the fact that colonies containing heterozygotes can be found, particularly in disturbed areas, implies that colonies are sometimes founded by unrelated females. The social structure of cooperative species -- colony foundation by a small number of females followed by inbreeding -- may have led to the observed reduction in allozymic variation in cooperative Anelosimus relative to solitary species.

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Female Biased Sex Ratios in Social Spiders: a Result of Group Selection?

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Among the group of spiders known to exhibit some form of social behavior, 15 species, representing four different families¹, can be singled out for having developed permanent sociality and for the possession of traits (communal brood care, non-overlapping generations and lack of reproductive castes) that define them as quasisocial. Unlike parasocial species (i.e. *Cyrtophora citricola*, *Metabus gravidus*, *Pholoponella republicana*, etc.), these spiders maintain a single common web used as retreat as well as for prey capture and feed on the prey communally. And unlike subsocial species (i.e. *Anelosimus studiosus*, *Stegodyphus lineatus*, etc.), individuals reaching adulthood continue to cooperate in the activities of the colony and remain together throughout their lives. As a consequence, the same nest is occupied by successive generations, the colonies therefore being potentially perennial.

Highly female biased sex ratios are the striking common feature of these species. The bias, as demonstrated for *Anelosimus eximius* (Aviles 1986) and suggested for other species, is not due to differential mortality of the sexes during the age of reproduction and it is already present as a tertiary and probably primary sex ratio². This apparently strong correlation between quasisociality in spiders and the violation of Fisher's principle has not been yet given enough attention as an evolutionary problem demanding explanation.

The explanation suggested for *A. eximius* (Aviles 1986) is now extended to all quasisocial spiders. Colonies of these species, because of permanent sociality, absence of migration between colonies, and a system of proliferation by budding, swarming or dispersal of gravid females from a single parental source², occur in nature as isolated population units whose integrity and that of their descendants is maintained through time. It is noted that in a system where males are capable of multiple matings, as in quasisocial spiders, a preponderance of females has an evident correlation with an increased growth rate of the colony. Since larger size of quasisocial spider colonies seems to be associated with higher survivorship and proliferation², a preponderance of females would represent an advantage to the colony as a whole. Selection within colonies would continue to favor the production of even sex ratios, but this process would be counteracted by selection at the higher level, the isolation of colonies being complete enough to prevent the spread of any even-sex ratio alleles. Female biased sex ratios in quasisocial spiders may be the evidence that their population structures have met the conditions under which the good of the group may override the advantage of the individual.

¹ *Agelena consociata* and *republicana* (AGELENIDAE), *Aebutina binotata*, *Mallos gregalis* (DICTYNIDAE); *Stegodyphus dumicola*, *mimosarum*, *sarasinorum*, *simoni* and *socialis* (ERESIDAE); *Achaearanea disparata* and *wau*, *Anelosimus domingo*, *eximius*, *lorenzo* and *rupununi* (THERIDIIDAE).

² See references in Aviles 1986.

Aviles, L. 1986. Sex ratio bias and possible group selection in the social spider *Anelosimus eximius*. *Am. Nat.* 128:1-12.

Comparative Social Structure of Yellowjacket Wasps and Fire Ants as Revealed by Genetic Studies

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The theory of kin selection focuses attention on patterns of relatedness and reproductive roles in insect societies. Despite its theoretical importance, knowledge of the social and genetic structures of insect colonies remains fragmentary, particularly outside the ants (see e.g., Pamilo, 1982, Crozier *et al.*, 1984). The task at hand, then, is to comparatively study these attributes in a diversity of taxa in the hope that any patterns emerging may shed light on the role of kin selection in the origin and subsequent development of insect sociality.

MATERIALS AND METHODS

Electrophoretic markers were used to study colony genetic structures in two social forms of the fire ant, *Solenopsis invicta*, and in two yellowjacket wasp species, *Vespula squamosa* and *V. maculifrons*. Estimation of relatedness from genetic markers follows Pamilo (1984).

RESULTS AND DISCUSSION

Colonies of *S. invicta* in the U.S. are predominantly monogyne (M), however, several discrete populations containing polygyne (P) colonies are known. Colonies of the M form are founded claustrally following nuptial flights, while colony reproduction in the P form appears to occur via budding. Details of the mating systems are unknown. Genotypic analyses of alate queens, workers, and males from over 100 M colonies reveal that these are simple family units headed by singly-inseminated queens who produce all colony members. Such a social structure should result in high relatedness among female nestmates (0.75), as was confirmed by regression estimates ($b=0.714$; Ross and Fletcher, 1985a).

Because multiple functional queens occur in P colonies of *S. invicta*, nestmate relatedness may be reduced; however, if these queens are full sisters and inbreeding is common then relatedness may be high. Analyses of queens from 30 P colonies indicate that nestmate queens are not full sisters (nor are they half sisters since P queens also are singly inseminated). Indeed, their relatedness is indistinguishable from zero, as is relatedness among worker nestmates. Evidence that the entire P population under study does not comprise an extended kin group descended from a few colonists comes from studies of allelic diversity at the sex locus (Ross and Fletcher, 1985b). The results suggest that close kin do not occupy individual nests and that considerable gene flow likely exists among nests in P populations of this species.

With few exceptions, yellowjacket wasps in temperate areas form monogyne colonies founded haplometrotically in spring by queens which mated the previous fall. Genotype distributions of females from 37 colonies indicate that multiple mating by foundresses is the rule for both *V. squamosa* and *V. maculifrons*. Polyandry results in relatedness among female nestmates of 0.403 and 0.320, respectively (Ross, 1986).

The possibility that sperm from various mates are used non-randomly by monogyne queens is a crucial consideration in assessing the importance of kin selection (Page, 1986), yet has not received thorough study in natural colonies of any species. Sperm utilization in the two yellow-jacket species was studied by periodically sampling workers from 8 colonies. Haplotypes of sperm used for fertilizations are inferred from worker and foundress genotypes. Utilization of sperm from different males appears largely random through the season, as evidenced by small differences between the insemination-effective and short-term effective numbers of matings (see Page, 1986). Thus workers present throughout the season have a similar moderate level of relatedness to the queens which are eventually produced (Ross, 1986).

Theoretical models indicate that reductions in worker inclusive fitness effects due to multiple mating by queens can be compensated for by direct production of males by workers, and several authors have suggested that this is the rule in queenright *Vespula* colonies (see Ross, 1985). Analyses of male haplotypes in 23 colonies of *V. squamosa* and *V. maculifrons* with appropriate genotype arrays reveal that all males in queenright colonies are sons of the foundress. On the other hand, substantial numbers of males of worker origin can be identified in queenless colonies. Thus queen control over worker reproduction is strong in these species and workers in queenright colonies have a zero direct component to their inclusive fitness.

These comparative data and results from other studies indicate that while colonies of some highly social Hymenoptera have high levels of nestmate relatedness, others have intermediate or low levels. Thus the evolutionary stability of advanced social structures in some groups cannot be fully explained by kinship factors alone, and alternative explanations emphasizing the constrained options of workers, maternal manipulation, and extrinsic factors must receive further consideration. If multiple mating and polygynous social structures are universally derived traits, then the origin of sociality may be mediated by kin selection, while its subsequent development has become uncoupled from the necessity for high relatedness yet constrained by the specialized morphologies and behaviors of the interactants.

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Polyandry in *Lasius* Ants

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The frequency of multiple mating of *Lasius niger* and *L. alienus* queens in natural populations was studied with genetic variation at polymorphic enzyme loci. Horizontal starch gel electrophoresis was performed on combinations of inseminated queens, sampled after a nuptial flight, and their first offspring, the nanictic workers. Two methods were applied on the genotype patterns to estimate the frequency of multiple mating: direct deduction, with a gene-frequency correction, and an ANOVA method. The results clearly show between-population differences and the adaptive significance of multiple mating in relation to habitat heterogeneity, resource availability and sex ratio theory is discussed.

An Unexpectedly High Female Bias to the Sex Ratio in a Temperate Population of *Halictus ligatus* - a Primitively Social Sweat Bee

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INTRODUCTION

Data from investment ratio studies of social insects are of great importance in testing theories which seek to explain the origin of eusociality. Under kin selection theory, an investment ratio of close to 1:1 is always preferred by queens, whereas the workers' preferred ratio depends upon the proportion of males that are worker-produced. Thus, if workers produce all of the haploid eggs then they will prefer a ratio close to 1:1. However, if workers are sterile they will prefer a ratio of 3:1 investment in females to males (Trivers and Hare, 1976, Science). Here, I present data from a field study of *H. ligatus* in which an investment ratio of 3:1 was obtained even though the workers seem to be responsible for laying most, if not all, of the haploid eggs.

METHODS

A large aggregation of *H. ligatus* nests was studied in detail in southern Ontario in 1984. Over 130 nests were excavated during the course of the summer. Data were analysed in 10 day blocks with day 1 being the first day that pollen foraging was observed at the aggregation site. Female bees were dissected: developing and resorbing oocytes were differentiated & the fraction of a fully developed oocyte present in each ovariole was estimated. Bee head widths were measured and mating condition noted. Sex ratios were estimated from both pupal and pollen ball counts (male and female reproductive producing pollen balls differing in size and shape). Investment ratios were obtained from the sex ratio estimates and pollen ball weights.

RESULTS

Analyses of ovarian condition indicated that the ovarian development of workers decreased monotonically during the summer provisioning period, whereas the proportion of them that had mated increased dramatically during this time. Queen ovarian development remained high during this phase. Estimates of oviposition success (obtained by subtracting the number of resorbing fully developed oocytes from the number of healthy ones in a cohort of queens and workers within a ten day period) indicated that workers may lay all of the eggs in the earliest period of reproductive production, but that thereafter, the queens produce most or all of the eggs. In total, workers produce approximately 39% of all reproductive brood eggs. Pupal counts give an estimate of approximately two females

for every male in the reproductive brood. When differences in the weight of the provision masses are taken into consideration, this becomes an investment ratio of approximately 3:1.

DISCUSSION

Worker ovarian development is at its highest when the nests are producing males and few workers are mated. The fact that workers seem to produce 39% of all reproductive brood eggs and that 34% of this brood is male indicates that they produce most, if not all, of the haploid eggs. Under this condition, kin selection theory predicts investment ratios of approximately 1:1 - whereas a value of 3:1 was obtained. Clearly alternative explanations for this female biased sex ratio have to be considered. Four other hypotheses have been suggested that might explain this: 1) the caste plasticity hypothesis (Strassmann, 1984, Evolution), 2) local mate competition (Hamilton, 1967, Science), 3) intra-demic group selection (Wilson and Colwell, 1981, Evolution) and 4) cyclic inbreeding (Stenseth, 1978, Oikos).

The colony cycle exhibited by *H. ligatus* does not fit the requirements of the caste plasticity hypothesis and the mating behaviours observed make local mate competition an unlikely explanation. It is believed that the female biased sex ratio found in this study is best explained in terms of the population structure and population dynamics of this bee.

H. ligatus (and many other sweat bee species) nests in low successional stage habitats where there is sparse vegetation and bare patches of soil. Thus, local populations are likely to be quite temporary, dying out as the vegetation grows too dense. This population structure may fit the requirements of the structured deme model allowing intra-demic group selection to apply. Wilson and Colwell list several factors that help promote female biased ratios within the structured deme model - thus, small initial group size, mating before dispersal, poorly regulated population size and a high variance in reproductive success all increase the likelihood of a female biased sex ratio occurring. All of these factors may operate in sweat bee populations.

Additionally, aggregations of halictine nests may be initiated by a small number of mated females (perhaps only one) and population crashes are to be expected as a result of vegetation change and the build up of predators and parasites; such dramatic population crashes have been observed (Knerer, 1973, Zool. Anz.). Under these conditions frequent but irregular periods of inbreeding may be expected, indicating that Stenseth's hypothesis may apply.

Clearly the female biased sex ratio observed here requires corroboration from further studies of sweat bees, nonetheless, it is clear that studies of the population structure and population dynamics of these bees are badly needed.

The Empirical Analysis of Sex Allocation in Monogynous Ants

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In 1976, Trivers and Hare presented a list of dry-weight sexual investments for 21 monogynous, non slave-making ants and claimed that the regression of sex-ratio on weight-ratio in this data set demonstrated the operation of worker control as expected from kin-selection. They were severely criticized, however, by Alexander and Sherman (1977), arguing in favor of local mate competition to explain gyne-biased sex investments in eusocial Hymenoptera. In spite of this criticism, theoretical studies rapidly and successfully concentrated on models of outbreeding populations, in which genetic relatedness and worker control were crucial parameters (Uyenoyama and Bengtsson, 1981; Pamilo, 1982 and references cited).

However, progress on the empirical side appeared to be much slower. For monogynous ants, the data set used by Trivers and Hare was not even doubled over the last decade. Recently, Nonacs (1986) summarized a number of arguments against the prevalence of local mate competition in monogynous ants, but his recalculations of the mean sex-investments in this group could yet only be based on data from 33 species. This low number would not be a problem if the 33 separate estimations of weight investment in ant sexuals were unbiased and equally accurate. As I will show below, this is apparently not so, and we must thus be very careful in drawing general conclusions from such data sets.

Investments in the gyne- and male caste should ideally be estimated by multiplying the number of sexual individuals with the estimated cost of an average gyne and male (investment-ratio = numerical sex-ratio \times cost-ratio). In practice, however, the mean gyne/male cost-ratio is usually estimated by weighing a sample of ant sexuals (Trivers and Hare, 1976; Nonacs, 1986). In doing so, it is assumed that body-weight is directly proportional to the cumulative amount of energy which was invested by workers between the egg stage and nuptial flight.

There are at least two good reasons to doubt if this assumption is justifiable throughout monogynous ants: At first, interspecific variation in sexual dimorphism is large, which affects the percentage of invested energy used for assimilation and respiration, respectively, due to the fact that smaller individuals tend to have higher metabolic rates. In species like *Lasius niger* and *L. flavus*, having extreme degrees of sexual (weight) dimorphism, metabolic rates ($\mu\text{l O}_2/\text{mg/h}$) were found to be more than twice as high in males than in gynes (Boomsma and Isaaks, 1985; Nielsen *et al.*, 1985). This difference implies that body-weight underestimates the energetic investment in *Lasius* males and that an overall correlation between the degree of underestimation and sexual dimorphism might be expected.

Secondly, mature gynes and males differ in water content and energy content per mg dry weight as adaptation to their very different future lives. This point can also be illustrated with data from *Lasius* ants. Males appear to contain 65-70 % of water throughout maturation, whereas gynes decrease from ca 70% at eclosion from pupa to 40-45 % at maturity

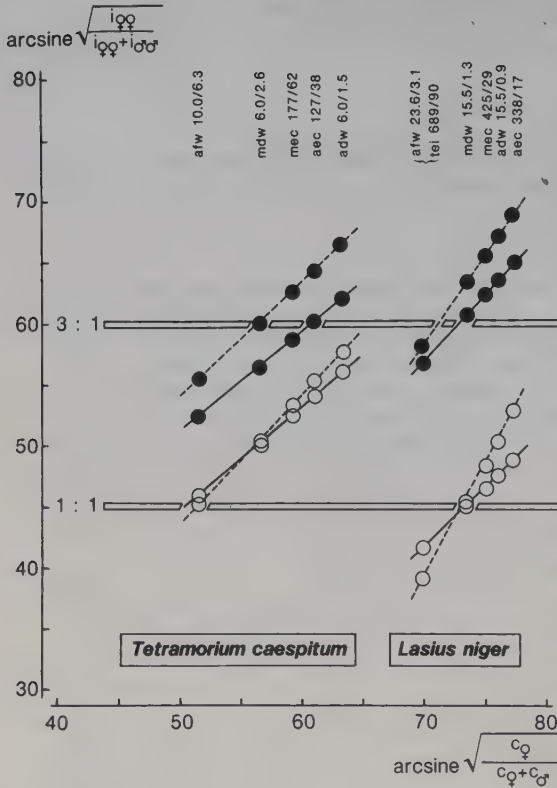


Fig. 1. -- Relative investment in gynes as function of the estimated cost of one gyne relative to one male, for *Tetramorium caespitum* and *Lasius niger* in the optimal habitats Ditch and Strandvakte (black symbols) and marginal habitats Knoll and Kobbeduinen (open symbols), respectively. Both mean investment per colony (solid lines) and pooled investment per population (dashed lines) are plotted. Before calculation of means, frequencies were angular transformed to get more normal distributions. Data on sex-ratios and cost-ratios were derived from Peakin (1972), Brian (1979), Boomsma *et al* (1982) and Boomsma and Isaaks (1985). The latter are given in the top of the figure in Joules or mg: a=adult; m=maximum; t=total; f=fresh; d=dry; e=energy; w=weight; c=content; i=investment. The theoretical optima 3:1 and 1:1 are indicated with horizontal bars.

(Nielsen *et al*, 1985; Boomsma and Isaaks, 1985; Boomsma, unpublished). At the same time, gynes of these ants, and of less dimorphic species like *Tetramorium caespitum* (Peakin, 1972), substantially increase in weight and (due to fat accumulation) energy content, but males do not. Fat reserves require more energy to be built up, but do not metabolize themselves and so, the total investment in gynes will be lower than expected from weight data for the respiration component but higher than expected for the assimilation component. Both errors work in opposite directions, but it seems unlikely that they will offset each other completely. Accordingly, parameters like fresh weight, dry weight and energy content as used in different studies on monogynous ants (cf Nonacs, 1986) may be expected to produce very different estimations of the relative investment in gynes and thus to be not comparable at all.

The dependence of estimations of relative investment in the gynecaste on the type of gyne/male cost-ratio used is plotted in Fig. 1 for two monogynous ants for which a number of alternative cost-ratios were available in the literature. For both *Tetramorium caespitum* and *Lasius niger*, having a moderate and extreme degree of sexual dimorphism, respectively, the range of possible outcomes is so large that investments as different as 1:1 and 3:1 can be derived from the same set of numerical data. The choice of either some dry-weight or some fresh-weight cost-ratio, and the deliberate or accidental inclusion of only mature gynes in the former and a mixture of immature and mature gynes in the latter is sufficient to produce this difference.

Weight investments are not only inaccurate, however, but also

biased, since they seem to overestimate energetic investments to a degree proportional to the specific degree of sexual dimorphism. Such artifact, expected already from the data summarized above, was argued elsewhere to be the most plausible explanation for the positive correlation between investment in the gyne-caste and gyne/male weight ratio that was found to occur in Nonacs' (1986) data set (Boomsma, submitted). Two more reasons for literature data on the proportional investment in gyne-castes to be overestimations were analyzed in that paper as well: Firstly, several records combine sex-ratio data from different populations in one estimate of the mean, inducing a bias when those populations differ in overall productivity. As shown in Fig. 1, pooled estimates tend to give more gyne-biased means than means per colony. Secondly, the number of colonies in the samples used by Nonacs (1986) appeared to be negatively correlated with the estimated investment in the gyne-caste. Both types of bias are apparently due to the fact that larger and more conspicuous colonies, which tend to produce a more than average gyne-biased investment, are overrepresented in small samples (Boomsma, submitted).

According to the arguments presented, it seems obvious that weight estimations of sexual investment across species are unsuitable for testing the validity of the genetic relatedness hypothesis in monogynous ants. Allozymatic estimation of genetic relatedness in different conspecific populations can provide more precise expectations of sex-investment ratios, which can be directly compared with the investments actually observed.

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Selection in Social Insects: The Superorganism Concept

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That natural selection favors the development of eusociality in north temperate zone honey bees (*Apis mellifera* L.) is made amply evident by the wide geographical distribution and perennial nature of the colonies of the species. Their successful exploitation of habitats into which they have been introduced reveals their remarkable adaptability. Groups separated for sufficient lengths of time have developed into distinct geographic races which are manifestations of different genotypes adapted to different environments (Louveaux 1969). Colony survival depends on a complex of interactions by members within the context of the social group which itself resembles an organism in its response to environmental stimuli, and early in this century was referred to as a "superorganism." Use of this term infers that the colony as a whole is a discrete unit, and as such, is subject to selective processes. If selective pressures operate at the level of the social group, group effects that are not evident in isolated individuals should be evident. Such group effects must also show genetic variation on which selection can act, heritability, and group-specific differences in fitness (Lewontin 1970). Colony-level selection would also imply that colonial fitness is enhanced even at the expense of individual sacrifice. In other words, individuals would be selected which maximize the reproductive success of the colony (Crozier 1979).

Honey bees show a number of altruistic behaviors that seem to fit these criteria such as the act of stinging which protects the colony but results in loss of life to the individual, or the behavior pattern of individuals removing themselves (and their genes) from the colony if sick, poisoned, or dying. These behaviors enhance the colony fitness at individual expense. However, close examination shows that these activities also may enhance the fitness of closely related nestmates demonstrating inclusive fitness (Hamilton 1964, 1972), and are not, then, clear indications of colony-level selection.

I report here empirical data showing sets of behaviors which decrease (or at least do not increase) individual advantage, but clearly increase the reproductive success of the group. The first set of data shows a metabolic response tightly coupled with defensive behavior which is released by exposure to alarm pheromone (Southwick and Moritz 1985). The magnitude of the response is highly dependent on the number of bees in the group. However the relationship is unexpected in that each individual shows an increased response with increased size of the group. There is no individual advantage for greater individual reactions when the group is large. In fact, it would seem that any advantage to greater individual response would occur in the smallest groups (causing a relatively more vigorous response to danger). Groups of sisters show the same magnitude of

response, but the responses of other groups vary according to genetic relationship. Heritability is demonstrated in at least first generation offspring, and there is no response shown by individuals.

A second colonial genetic trait subject to selection is the group size dependence of oxidative metabolism (at cold temperatures when clusters form). Individuals die at cold temperatures. Individuals in groups show progressively lower rates of metabolism as the group becomes larger. The total cluster metabolic rate is much less than the sum of the expected rates of individuals comprising the cluster (Southwick 1985). At extremely low temperatures, individuals on the outside of a cluster may die but continue to contribute insulative value to the cluster. At environmental temperatures normally encountered in winter in north temperate habitats (e.g., averaging ca. 2°C in Frankfurt, Germany), there is an optimum colony size (17000 individuals) below which survival is metabolically more costly. Yet, at higher temperatures (15°C) smaller colonies cost considerably less to maintain (but individuals within the smaller colonies have higher metabolic costs). The bee race best adapted for cold but variable winters is not that with the largest population and lowest individual cost (e.g., *A. m. ligustica*) as would be expected from individual selection, but instead a race with a moderate winter population and higher individual costs (e.g., *A. m. mellifera* or *A. m. carnica*) (F. Ruttner, personal communication). The colony size/metabolic cost relationship functions as an entity for selection highly dependent on long-term environmental temperature.

These features of the intact social group are different than those shown by isolated individuals, and clearly provide for colony differences in fitness, genetic variation, and heritability. Thus, the cluster itself, as well as the individual, is a fundamental characteristic of the species and a basis for selective success in evolution.

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Does Worker Dominance Reduce Group Fitness of Honeybees (*Apis mellifera capensis* Escholtz)?

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In highly eusocial insect colonies like those of honeybees, *Apis mellifera*, there is a clear dominance hierarchy. The queen is the reproductive dominant individual and the worker bees do not produce offspring. In queenless colonies, however, a worker hierarchy is established and dominant workers may develop ovaries and start laying eggs. The individual worker dominance is characterized by a rapid development of the ovaries, a high production of eggs and of 9-oxo-2-decenoic acid, the main component of the queen substance. Also, dominant worker bees are always fed by subordinates and rarely offer food to other nestmates (Moritz and Hillesheim, 1985). These characters are genetically fixed and their heritabilities range from 27% to 89%. Therefore, dominant workers have a greater individual fitness. Consequently the gene frequency of "dominance" alleles in the population should increase and finally go towards fixation. In colonies of natural populations, however, we find well established hierarchies with strong genetic variance components for dominance behaviour.

Group selection may explain this phenomenon, such that pure colonies of dominant worker bees could have a lower colony fitness. To test this hypothesis we show in this study whether groups of subordinate and dominant worker bees differ in collective behavioural phenotypes.

MATERIALS AND METHODS

Hoarding behaviour and the alarm response to isopentyl acetate (IPA), a major component of the alarm pheromone, were tested. From a breeding project subordinate (S), dominant (D) and hybrid (DS = D queen was inseminated with S semen) workers were available. For the hoarding behaviour 100 newly hatched bees - S, D and mixed groups (S+D) were caged in screened containers for eight days. The cages were supplied with an empty piece of comb (8x8 cm). The bees were fed with a 2 M sucrose solution, water and pollen ad libitum. The test cages were placed in an incubator at 35°C, 75% r.h. and constant dark. In order to determine the amount of stored sugar, the comb was weighed before and after the test period. Using a light refractometer we measured the sugar concentration of the stored "honey" in the cells, and we were able to calculate the actual sugar solution hoarded.

For testing the alarm response, groups of 40 newly emerged workers were tested. Each group was made up of only subordinates, only dominants, or mixed groups of both (S+D) and hybrids (DxS). After 4 days each test group was placed in an airtight 100 ml container with a constant airflow. The test group was exposed to 10 seconds of IPA in the stream of air by a timer controlled T-valve. The increase in the CO₂ production was measured by a CO₂ Infrared analyzer.

RESULTS

Figure 1 shows the results of the hoarding behaviour. There is a significant difference among the three types of groups (ANOVA; $F=3.232$; $p=0.05$). In this test the subordinate groups hoarded the most, the dominants collected less and the mixed groups were in the middle.

In Figure 2 the reaction of the tested groups to the IPA stimulus is shown. Also for this test there is a highly significant difference among the tested groups ($F=8.94$; $p\leq 0.01$). The subordinate groups showed the strongest reaction; the dominant groups remained calm and the mixed groups as well as the hybrid groups showed a medium reaction.

Fig.1

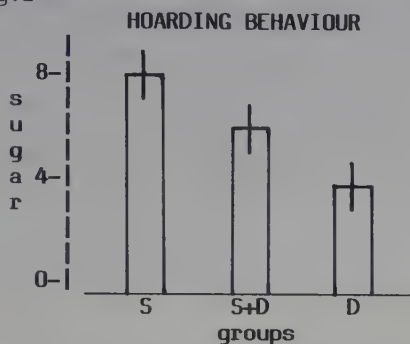


Fig. 2

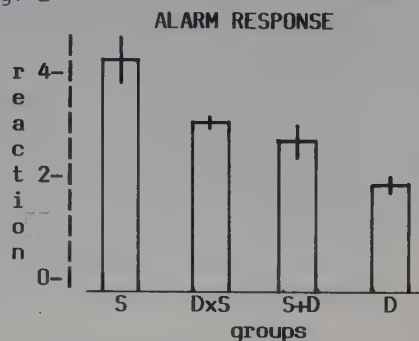


Fig. 1 Results of hoarding behaviour. S = subordinates; D = dominants; S+D = mixed groups of S and D. Y-axis = hoarded sugar in the comb (g).

Fig. 2 Results of the alarm test. X-axis = same as Fig. 1 and DxS = hybrid group of D and S. Y-axis = CO₂ production (µlCO₂/bee/min)

DISCUSSION

Pure groups of subordinate workers show a different reaction in hoarding behaviour and alarm response than mixed groups or groups of dominant worker bees. There are linear phenotype interactions in both sets of results. Individual dominance level of worker bees definitely has an effect on the expression of group phenotypes.

Investigations of the colony fitness on the reproductive level, as brood production, drone and queen rearing should be done to improve our understanding of the colony composition and the basis for selection in social populations.

Acknowledgement: I thank the Deutsche Forschungsgemeinschaft (DFG) for giving financial support (grant Ko 400/6-1).

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Genetic Parameters of Populations of Social Bees

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Data are produced that allows estimating the number of xo-heteroalleles of Melipona compressipes fasciculata as being equal to 17.5 which does not differ significantly from Apis mellifera ($n = 18.7$). Emphasis is given to the fact that the lethal equivalent estimate for Apis mellifera (1.29 to 1.36) does not differ from the one found for meliponids (1.08 to 1.14). These equalities are supposed to be due to similar evolutionary pressures that sex determination and eu-social life exercise upon the genetic parameters of the populations of these bee species.

Sex Ratios and Evolution of Eusociality

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A number of theoretical models for the evolution of eusociality exist (see Pamilo, 1984). It has, for instance, been suggested that the ability to bias the sex ratio may have facilitated the evolution of worker behaviour in Hymenoptera. This biasing can also create a sex ratio conflict between the mother and her worker offspring (e.g. Crozier, 1979). The aim of the present study is to examine how sex ratio biasing can affect worker evolution in simple genetic models of 'offspring altruism' and 'parental manipulation'. The study is based on computer simulation of a bivoltine life cycle. A female offspring in the summer generation has two options: to mate and become a lone foundress or to stay and raise a brood produced by the spring foundress (her mother). The model is deterministic and aims to examine how the evolution of worker behaviour can be affected by biasing the sex ratio.

The model allows independent genetic determination of the sex ratio among the offspring of the spring foundresses in the summer generation (S1), among the offspring of the 'selfish' females in the autumn generation (S2), and among the offspring raised by the workers in the autumn generation (S3). If the workers can bias S3, they make it female-biased. This gives mating advantage to the males produced by the selfish summer females and worker behaviour does not reach a high frequency. If selfish summer females can bias S2, they compensate the female-biased S3 by producing sons. This interaction between the two types of females can lead to a faster spread and a higher equilibrium frequency of the worker behaviour. If the spring foundresses can bias S1, they tend to make it female-biased when part of their daughters need not mate because they become workers, and part of their daughters need not mate because they produce only sons. When both queens and workers can affect S3, the model predicts that the nests specialize in producing either females or males, and finally $S3=0.5$ (fifty-fifty ratio). This conflict over the sex ratio drives the evolution of worker behaviour more effectively than mere worker control. The threshold value of worker efficiency which allows the evolution of worker behaviour is lower in parental manipulation than in offspring altruism.

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Macrogeographic Genetic Variation in the Sweat Bee *Lasioglossum (Dialictus) zephyrum*

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Understanding the population structure of a social species requires information on both microgeographic and macrogeographic levels. Studies of social insects at the macrogeographic level are rare and confined to ants where the presence of important differentiation has been reported in several species. We report here the results of an extensive study of the distribution of allozymes over a wide area for *L. zephyrum* a primitively eusocial sweat bee.

Samples of approximately 40 females, one per colony, were collected from 37 aggregations along creeks in three separate drainage systems in New York and Pennsylvania. All females were examined as described in Kukuk and May (1985) for 12 polymorphic, enzyme coding loci.

Within nesting aggregations no deviation from Hardy-Weinberg equilibrium frequencies or linkage disequilibrium was detected. No evidence of genetic differentiation among aggregations within creeks was detected. Genetic distances for aggregations within creeks are very near zero. There is, however, significant differentiation among creeks within drainages and among drainages.

Genetic diversity does not appear to be very great among the aggregations of *L. zephyrum* examined. Aggregations, which may contain hundreds or thousands of colonies located within 5 miles of one another along the same creek, appear to be undifferentiated from one another suggesting that nest aggregations are not isolated populations. Significant heterogeneity does exist among creeks suggesting that some degree of isolation occurs within drainage systems.

The major effects of geographic variation in *L. zephyrum* only appear when several creeks or drainage systems are included in an analysis, suggesting that either (1) models for the evolution and maintenance of eusocial behavior in halictine bees should concentrate on models involving panmixia or (2) population viscosity important for the evolution of eusociality occurs at the microgeographic level, within nest aggregations. Effective population size was estimated to be 1.2×10^3 .

Kukuk, P. F., and B. P. May. 1985. A re-examination of genetic variability in *Dialictus zephyrus* (Hymenoptera: Halictidae). *Evolution* 39:226-228.

Restriction Site Polymorphisms in Mitochondrial DNA of American and Africanized Honey Bees (*Apis mellifera*)

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This study demonstrates polymorphisms in mitochondrial DNA (mtDNA) restriction sites of American and Africanized bees. MtDNA was prepared (Brown 1981) from bees from a domesticated (USA1) and a feral hive (USA2) from Washtenaw Co. Michigan, USA, and from frozen tissue from three hives of Africanized bees (BRZ1, BRZ2, BRZ3) from the apiaries of the University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brasil. Each sample of mtDNA was prepared from flight muscle tissue of 100 adult workers. mtDNA samples were digested with 4-base (Mbo1 and Hinf1) and 6-base (Ava1, Bcl1, EcoR1, Hinc2, Hind3, Nde1, Pst1, Pvu2, and Xba1) restriction enzymes and the fragments were separated on 1.2% agarose and 4% polyacrylamide gels (Brown 1980; Ferris et al. 1981). Maps of restriction sites generated by 6-base enzymes were constructed for each hive. The total length of the mtDNA molecule estimated from the lengths of 6-base restriction fragments from USA1 is 16,450 base pairs. The nine 6-base enzymes produced 23 cuts in USA1. Africanized differed from American bees by loss of four restriction sites (a Bcl1, EcoR1, Nde1 and Xba1 site) and in total length of the mtDNA molecule (mtDNA from Brazilian samples was approximately 400 base pairs larger than that of USA1). BRZ2 and 3 differed from BRZ1 by the gain of one new Bcl1 site. USA2 differed from USA1 by loss of one Xba1 site and gain of one new Nde1 site. Sequence divergence (Nei and Tajima 1983) among the five samples was estimated using the cleavage maps. By this analysis there was approximately 2% sequence divergence between Brazilian samples and American samples (from 1.8% between USA1 and BRZ1 to 2.9% between USA2 and BRZ2 and 3). There was approximately 0.7% divergence or less within population samples (0.7% between USA1 and 2, 0.4% between BRZ1 and BRZ2 & 3, and none between BRZ2 and BRZ3).

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Isolation of Homeo Box Sequences from Honey Bees

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To study genes that control early embryonic development of the honey bee, we have isolated six homeo box containing genes using homeo box sequences of *Drosophila melanogaster*.

A honey bee genomic library in the EMBL 4 vector was constructed and screened with *Antennapedia* and *engrailed* homeo box sequences of *Drosophila melanogaster*, as well as with a *Drosophila* homeo box located at 99B which is genetically not yet defined. Sequence analysis of all homeo boxes revealed that all six genes isolated have strong homology to their corresponding *Drosophila* genes, i.e. *Deformed*, *Sex combs reduced*, *Antennapedia*, *Infra-abdominal 2*, *engrailed* and 99B as we called the not yet identified *Drosophila* homeo box. The region of homology includes the homeo box itself and sequences 5' and 3' of the homeo box. Homologies outside the homeobox are 4-29 amino acids long depending on the gene. The degree of amino acid homology varies between 90% and 100%. To test if the isolated honey bee genes have similar functions as the corresponding *Drosophila* genes, in situ hybridization to sections of honey bee embryos was performed. First results with the clone H42 revealed a spatial and temporal equivalent hybridization in the honey bee compared to hybridization of *Deformed* in *Drosophila*. This fact is a strong indication for a similar function of both genes.

3.4 Evolution of Insect Societies

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The Epigenetical Origins of Insect Sociality

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Evolutionary theories of insect sociality deal extensively with the problem of explaining the spread and stable persistence of genes promoting the helping (or "altruistic") behavior of workers (for a review of genetic models, see Michod, 1982). It is now desirable to take full account of the fact that worker and reproductive phenotypes are usually facultative in nature: they are alternative expressions of a single genotype, with the phenotype adopted depending on developmental and/or behavioral circumstances (long referred to by students of social insects as "caste determination"). This lecture addresses the question of how such facultative adaptive responses are constructed during evolution.

Consideration of the reproductive cycles of solitary, primitively social (group-living but casteless), and eusocial (worker containing) wasps indicates that the facultative expression of a worker phenotype is derived from two condition-sensitive responses evidently widespread in solitary species, namely, broodcare in the presence of larvae and oosorption when oviposition is blocked (see Flanders, 1962; Bell and Bohm, 1975). Observations of the primitively social eumenid wasp *Zethus miniatus* Saussure showed that individual females usually behaved like solitary wasps, independently building cells, laying eggs, and provisioning and defending their own larvae one at a time. However, temporarily sterile individuals (broodless females lacking a mature ovarian egg) adopted the orphaned larvae of other females if any were available. This suggests that worker behavior can originate as misplaced parental care by an eggless female in the presence of orphans, and might occur with little or no genetic change in groups which have evolved under selection in other contexts (e.g., reuse of cells under selection for economy of construction activity, or mutualistic defense). Intragroup reproductive competition, likewise expected to be a common result of life in groups (see West-Eberhard, 1981), has evidently produced permanently sterile workers via the evolution of effective devices (including parental manipulation) for monopolizing oviposition ("queen control"). Control of nestmate oviposition by filling and defending cells may have been sufficient cause for the advent of worker sterility, since oosorption when oviposition is blocked is a widespread response in the Hymenoptera and other insects (Flanders, 1961; Bell and Bohm, 1971). At the same time, aggressive queen control could generate, as a side effect, a supply of orphaned larvae, the product of a negligent (non-foraging) dominant female ("queen") preoccupied with territorial domination of the nest. The hypothesis that a worker caste is readily derived from a solitary phenotype given life and competition in groups is supported by the finding that a worker phenotype can be produced in solitary bees (*Ceratina* species) experimentally forced to live in groups

(Sakagami and Maeta, 1986). Even if some worker traits in such species were to prove vestiges of an ancestral social state, these studies are important demonstrations of the ease of passing back and forth across the border between solitary and eusocial life (see also, Michener, 1985).

Kin selection (the genetic profit associated with aid to kin) undoubtedly plays an important role in the evolution of a sterile worker caste, which in all known cases occurs in kin groups. However, given the above considerations kin selection is not necessary to explain the origin of sterile workers, which can occur without positive selection of alleles for helping non-offspring. Rather, kin selection may act primarily to maintain facultative aid by affecting the evolution of regulatory mechanisms assuring that worker behavior is expressed only when likely to be adaptive. Other behaviors (such as surreptitious egg-laying, waiting for a future opportunity to reproduce, independent nest initiation, or fighting to the death) expressed as alternative patterns when more advantageous than worker behavior (West-Eberhard, 1981). Kin selection would thus play a role, not in the spread of "altruistic" alleles, but in the evolution of the regulation of the worker phenotype once it has originated as a side effect of selection in other contexts (e.g. favoring cell reuse or mutualistic defense, and control of nestmate oviposition).

By this "epigenetic" interpretation Hamilton's Rule for the operation of kin selection describes the switch point for the expression of the worker phenotype in subordinate individuals (West, 1967; West-Eberhard, 1975). Hamilton's Rule-- $K > 1/r$ --states that there is positive selection for helping behavior when the ratio of benefit to cost of aid (K) in terms of individual fitness is greater than the reciprocal of a coefficient of relatedness between donor and recipient (r). The epigenetic interpretation predicts the evolution of mechanisms permitting the evaluation of K and r , including use of cues indicating expected reproductive success and relatedness, as well as the setting (under natural selection) of threshold states of those cues likely to yield profitable worker behavior in particular species and situations. Such cues exist, e.g. in the form of dominance relations (proposed as an evaluator of K --see West-Eberhard, 1967), and kin recognition devices (evaluators of r --reviewed in Gadagkar, 1985). And there is some evidence (e.g., West, 1967; Noonan, 1981; Metcalf and Whitt, 1977 a and b; Strassman, 1981) that Hamilton's Rule is satisfied in nature.

Thus far, all of these field studies using social insects to test kin selection theory have examined the results of facultative alternative patterns and confirm the importance of kin selection in the evolutionary fine-tuning of a caste-determining switch mechanism. That is, they are tests of an epigenetic model concerned with expression of alternative phenotypes rather than of a genetic model concerned with competition among alternative alleles.

Evolutionary epigenetics calls for a synthesis of information and theory on development, genetics, phylogeny, and comparative behavior. It would aim to describe the likely phenotype transitions from ancestral to derived phenotypic states. Such an approach, in the tradition of Roubaud (1916) and Wheeler (1920), is not new to students of social insects. But incorporation of a developmental viewpoint (e.g., see Kennedy, 1966) has been neglected because of emphasis on genetic issues and failure to see precisely how the

insights of genetic theory should be related to information on development and ancestral phenotypes (see discussion following Kennedy, 1966; Craig, 1983 is a recent attempt at synthesis).

An epigenetic approach leads to a revised view of how major transitions occur during the evolution of social behavior, for example, suggesting that novel phenotypes such as eusociality and social parasitism can originate and be elaborated as facultative intraspecific alternatives rather than new branches on a phylogenetic tree (see West-Eberhard, 1986; and in press b for general discussions; Michener, 1985 on bees). Studies of social insects may help to illuminate the evolution of flexible behavior in general, especially given the wealth of information now available on the physiology, behavior, and environmental correlates of caste determination in species having different degrees of behavioral and morphological specialization (Nijhout and Wheeler, 1982).

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SYMPOSIUM

Development of Eusociality in Wasps with Reference to Polygyny

Organizer: Yosiaki Jtô

Introduction

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Modern theories on the evolution of insect eusociality are mainly based on the monogynous, matrifilial colony system, which is a rule in honey bees, stingless bees and temperate vespine wasps (Hamilton, 1964; Alexander, 1974). However, with the progress of surveys on tropical social insects, the number of reported cases of polygyny has increased especially in wasps and ants. In some cases, a polygynous colony changes to a functionally monogynous one where a dominant foundress monopolizes oviposition or selectively eats eggs laid by the subordinates. In other cases, however, multiple egg-layers coexist even after the emergence of progeny. In *Polybia* and some other neotropical genera which are closely related to *Polybia*, and the subgenus *Icariellia* of the genus *Ropalidia*, each colony has a large number of queens who are morphologically distinguishable from workers. Thus the role of polygyny in evolution of wasp eusociality must be clarified.

This is the reason I organized this symposium.

Of course the social structure, especially the number of egg-layers per colony, of a species or a local population is different from other species (Report in this symposium by Turillazzi for *Stenogastrinae*, Keeping and Crewe for *Belonogaster*, and Ito for *Polistinae*) or from other local populations of the same species (Strassmann and Queller for *Pollistes bellicosus*). But it is now recognized that, at least in some environmental or social conditions, wasp colonies can be truly polygynous, that is, having multiple egg-layers after emergence of progeny females. This fact suggests that mutualism (Lin and Michener, 1972) is relatively more important than kin-selection in the evolution of such a social structure.

Strassmann and Queller (this volume) reported that size and other measures of physical condition were not factors that caused females to join groups. A similar situation has recently been found in some ants (e.g. Peeters and Crewe, 1985 for a ponerine ant, *Ophthalmopone berthoudi*) and in artificially induced eusocial colonies of ceratine bees (Sakagami and Maeta, in press). Predation pressure and other risk of nest failure due to unpredictable environmental changes might be important factors which lead to group nesting (Strassmann and Queller, Keeping and Crewe, Ito, this volume).

Explicit explanation of the evolution and maintenance of multiqueen colony systems in some tropical vespid wasps as well as those in ants is not yet available, but I hope that readers can obtain some insight from papers presented in this symposium and in the symposium "Towards a unified reproductive biology of the Hymenoptera" (this volume). A symposium on a similar topic must, in my opinion, be held in the future, combining wasps, bees, ants, termites and aphids.

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Social Structure and the Determinants of Queen Status in the Primitively Eusocial Wasp *Ropalidia cyathiformis*

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Primitively eusocial wasps are of great interest to students of socio-biology because of the flexibility in social roles that an individual can adopt. This flexibility can be rather spectacularly illustrated using the common Indian species *Ropalidia cyathiformis* (Gadagkar and Joshi 1985). Here females eclosing on a nest have several options open to them. Some females leave the parent nest to start new, haplometric nests. Others also leave to found new nests, but pleometrotically i.e., they leave along with some workers. Yet other females stay on at the parent nest and eventually challenge the queen and take over the nest. Finally of course some females stay on at the parent nest and remain as sterile workers throughout their life. Prompted by the presence of such diverse reproductive strategies we have made a quantitative study of the behaviour of individually identified wasps. Even among the females staying back at the parent nest, multivariate statistical analysis of time activity budgets of individually identified wasps has revealed the presence of a behavioural caste differentiation into Sitters, Fighters and Foragers (Gadagkar and Joshi 1984). Here I report experiments designed to understand the biological and evolutionary significance of these behavioural castes.

MATERIALS AND METHODS

Naturally initiated post emergence colonies of *R. cyathiformis* were studied on the grounds of the Indian Institute of Science, Bangalore. Wasps were individually marked with spots of coloured paint, behavioural data were collected using unbiased sampling methods and multivariate and other kinds of data analysis were performed on a DEC 1090 Computer, all as described previously (Gadagkar and Joshi 1984). After sufficient data on a colony were collected, its queen was removed and the observations repeated.

RESULTS AND DISCUSSION

All the colonies studied showed a behavioural caste differentiation into Sitters, Fighters and Foragers both before and after the queens were removed. A typical pattern is illustrated in Fig.1. In conformity with previous results and in contrast to a related species *R. marginata* (Gadagkar and Joshi 1983), the queens of all the colonies were Fighters. In 7 out of 8 cases of removal or disappearance of a queen one of the subordinates assumed the role of a queen and began to lay eggs within about a week of queen removal. In every case the potential queen (the wasp that succeeds a removed queen) was a Fighter. As seen from Fig.1 the potential queen was remarkably different from all the other wasps in the colony and remarkably similar to the queen in her behavioural profile. In many cases the behavioural profile of that wasp who would eventually succeed the potential queen when the latter was in turn removed could be studied. Such a wasp is termed potential queen 2. The potential queen 2 was always a Sitter but not different from any other wasps in any discernable manner.

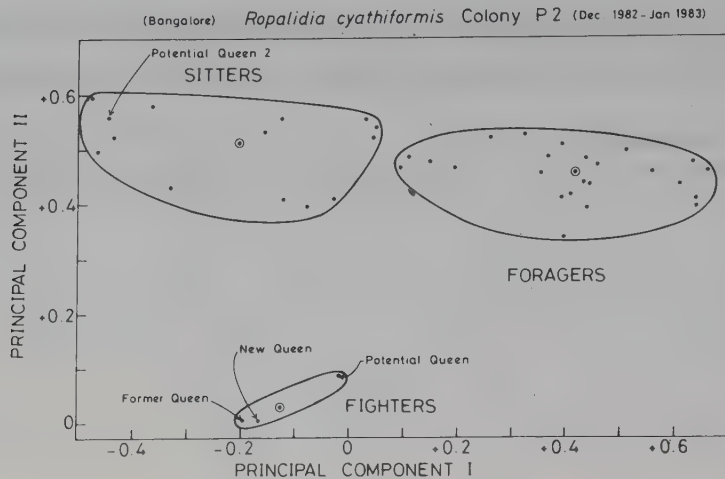


Fig.1.-Behavioural profiles of individually identified wasps in a *R. cyathiformis* colony analysed by principal components analysis. Each point represents the time-activity budget of a wasp (either before or after the queen was removed) plotted in the co-ordinate space of the first two principal components. The distinctness of the 3 clusters, Sitters, Fighters and Foragers is confirmed by the method of nearest centroid.

In 4 of the 7 cases the ages of the animals were known and in all of these the potential queen was not the oldest animal available. The four potential queens under discussion succeeded removed queens inspite of there being present 7, 10, 1 and 6 older females in their respective colonies at the time of queen removal. Among the females present at the time of queen removal the potential queens had the highest dominance rank and had shown the highest frequency of dominance behaviour in 6 out of 7 cases. The potential queens never brought food to the nest in 6 out of 7 cases and in the 7th case other females had brought food more often than the potential queen. Some of these results are in striking contrast to temperate zone wasps such as *Polistes exclamans* where high ranking but some of the oldest Foragers are known to be potential queens (Strassmann and Meyer 1983). In summary most colonies appear to have a well defined potential queen who can be predicted to become the next queen with reasonable certainty. The role of the potential queen appears to be fixed even in a healthy colony with an active queen.

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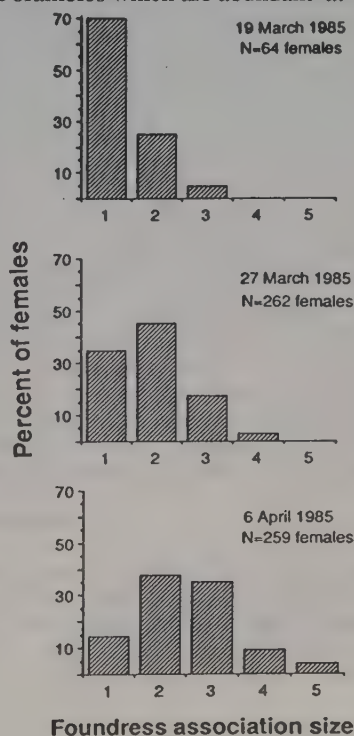
Constraints on Independent Nesting by *Polistes* Foundresses in Texas

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Every spring, female paper wasps (*Polistes*) begin new nests in which they lay eggs and then raise larvae. In many species, mated, potential queens join established nests where they function as workers, tending brood and submitting to aggressive attacks by the queens but not laying eggs. Here we investigate why these females altruistically aid others rather than building their own nests. We are particularly interested in the effects of two kinds of constraints on independent nesting: the condition of foundresses at nest initiation, and their prior experience before joining a nest. These variables are of interest because females that join nests may not be equivalent to those that initiate nests. If this is the case, then the reproductive potential of joiners cannot be estimated from the reproductive success of singly-founding females. This assumption has been critical to estimates of the advantage to group nesting in *Polistes* (e.g. Noonan 1981).

For this study we chose *P. bellicosus* because it is abundant in native prairies near Houston, and because differences among individuals are likely to be the principal constraints on solitary nesting. Nest site constraints, which may also favor group nesting, and may be important in other species of *Polistes*, are not likely to operate in *P. bellicosus* because it nests on bushes and brambles which are abundant at the study site. We followed 262 females for 19 days beginning on 19 March 1985. The first nests were initiated 4 days previously and new nests continued to be initiated until about 24 March. On 19 March, nests averaged 9 cells that all contained eggs (S.D.=4, N=54 nests). As females were encountered on new nests, we gave them a unique paint mark and measured their wing lengths. On 6 April, all but the largest female was collected from 26 nests and all but the smallest female was collected from 24 nests as part of another study. The ovaries and wings of these females and those from an additional sample of about 26 entire nests were measured. Females were divided into those still on their original nest (204 females), and those that were marked on a nest other than the one where they were collected (112 females).

Fig. 1. Foundress association size in *P. bellicosus* at 3 dates in early spring.



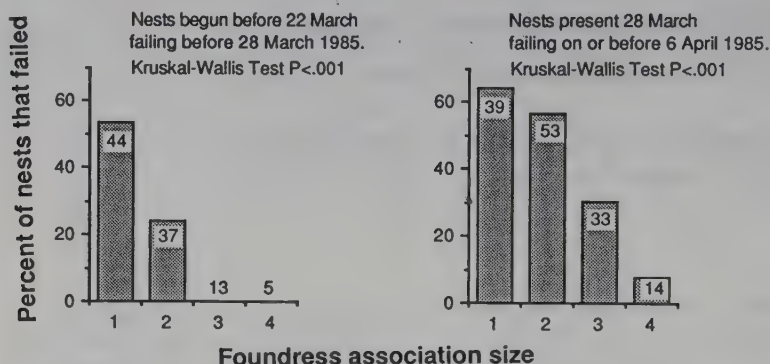


Fig. 2. Nest failure rates by foundress association size. Numbers of nests sampled are given on the bars.

RESULTS AND DISCUSSION

On 19 March 1985, 70% of females were alone on nests but by 6 April only 14% of females were alone (Fig. 1). This pattern did not result from differences in timing of arrival at the study site. Instead it appears that females first built a nest alone and then joined another nest if that nest failed. In all, 35% of females ($N=317$) were initially marked on a nest other than the one where they were collected. Females joining nests were not smaller than females on their original nest (Wilcoxon Test, $p > 0.2$), but they did have significantly fewer mature eggs in their ovaries (Wilcoxon Test, $p < 0.04$). This indicates that females usually joined as subordinates, but did not differ in size from females whose nesting attempts were successful.

Nests with multiple females were generally more successful than nests with only 1 foundress. Nest failure rates were significantly lower for groups (Fig. 2). On 6 April, nests with more than one foundress were significantly larger than lone foundress nests (multiple female nests: 21 cells, S.D.=6, $N=77$ nests; single female nests: 13 cells, S.D.=3, $N=23$ nests, Wilcoxon Test, $p < 0.001$).

Females usually joined sisters. Preliminary evidence from protein polymorphisms indicates that foundresses on the same nest are more closely related than 0.50.

Group founding in *P. bellicosus* occurs after attempts at independent nesting have failed. Females whose nests fail are not smaller than females that succeed. After failing however, joining (usually as a subordinate) is the alternative followed by most females, perhaps because they have lost time since the beginning of the season, or perhaps because their condition has deteriorated since nest initiation. In any case, joining close relatives on a nest that is more likely to succeed than is a single foundress nest may be an attractive alternative.

This study was conducted at Brazos Bend State Park under Texas Parks and Wildlife permit number 16-85. We thank C. Solis for field assistance, and D. Riskind, L. Fowler, and C. VanBaarle for their cooperation.

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Social Biology of the *Stenogastrinae*: Temporary Dynamic Reproductive Strategy in the Wet Tropics

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Recent studies on *Stenogastrine* wasps have shown the quite distinctive features of their biology and social behaviour and indicate that sociality in this group has probably evolved in an independent and quite original way (for an up to date list of references see Turillazzi 1986).

In most species of *Stenogastrinae*, nest foundation is strictly haplometrotic. Associative foundation is present in *P.alternata*, but interactions between associated females are rare and no sharp division of labour exists. Females from 7 biginic foundations showed fairly similarly developed ovaries on dissection (Turillazzi, 1985a).

In *P.nigricans serrei* the first individuals to emerge are usually females which remain on the parental nest helping their mother in rearing the larvae. Many of them spend a period of life acting as foragers. The first male usually emerges after the emergence of 3 to 5 females. In the first phases of the post emergence period the nest population coincides with the total population produced, but after a marked increase in the number of males some females begin to leave the colonies. The old foundress can be substituted by another female. Predation raids by *Vespa tropica* interest old and large colonies and can cause drastic destruction of the nest and brood and the abandonment of the colony by many individuals. Queen supersedure has been observed in *P.nigricans* (Turillazzi and Pardi 1982; Turillazzi, 1985b), *P.mellyi* (Yamane et al., 1983) and *L.flavolineata* (Hansell et al., 1982). Females of *P.nigricans* undergo ovarian development as they grow older, and mate within 20 to 50 days after emerging. Females with larger ovaries are fertilized before those with smaller ones, and reproductive potential differs between females of the same age (Turillazzi, 1985b). With the age of the colony the number of potential egg layers (PEL) present on the nests increases. In *P.alternata* the percentage of PEL females is about 51% while in *Liostenogaster* sp. it is only 22%. However the real contribution these females give to egg production in a colony has not yet been clarified and cannot be stated if potential polygynous colonies are actually monogynous. In *P.mellyi* only the dominant females have been seen laying eggs (Yamane et al. 1983) while in *P.nigricans* I observed that second rank females quite often have abundant yellow bodies in their ovaries as a sign of degenerating oocytes. In *L.flavolineata* females other than the dominant one can lay some eggs (Hansell et al., 1982).

The reproductive anatomical features of the females are in some species strictly related to their behaviour.

Dominance hierarchies (more or less linear and more or less structured) are present in P.nigricans serrei, P.mellyi, P.jacobsoni and L.flavolineata. In these species, dominant individuals can be recognized by their behaviour. Division of labour has been reported in many species. When full reproductive potential has been achieved three possible strategies seem to be open to females: 1) to leave the parental nest and found a new colony, 2) to remain on the parental nest hoping to substitute the leader female and trying to lay some eggs, 3) to leave the parental nest and try to usurp another colony.

Nest usurpation is widespread among Stenogastrinae. A great number of females of P.nigricans wander around looking for a new nest. Remaining on the parental nest could be preferred by females with a low reproductive potential or by females who are waiting to substitute the old foundress.

Regarding the origin of sociality in these wasps, group living was probably facilitated by their habit of nesting in suitable places to counteract the effects of pressure from predation. Another thing was possibly the "invention" of the abdominal secretion. This allowed efficient brood rearing with progressive provisioning and contact with the larvae and made it possible to use the larvae and the secretion itself as a storage centre for nutritive reserves for the colony, so that the nest became an "attractive place" for all kinds of adults. The evolution of social organization, owing to the necessity of maintaining colonial size within restricted limits, passed through the development of a temporal polyethism of the females. The period of permanence on the maternal nest could be increased by the intervention of a dominant mother or relatives. In this respect more detailed studies on as many species as possible are necessary.

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The Ontogeny and Evolution of Foundress Associations in *Belonogaster petiolata* (Hymenoptera: Vespidae)

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Tests of kin selection and parental manipulation theory using foundress associations of polistine wasps have generally estimated relatedness among cofoundresses and compared the reproductivity and survivorship of single foundresses vs queens and subordinates in multiple-foundress groups (e.g. Metcalf and Whitt, 1977, Dropkin and Gamboa, 1981, Noonan, 1981). Several studies have also investigated ecological factors selecting for single or cooperative colony foundation (Gamboa, 1978, Gibo, 1978). This paper tests the relevance of kin selection and parental manipulation theory in explaining the evolution of foundress associations in the polistine wasp *Belonogaster petiolata*.

RESULTS AND DISCUSSION

At Sterkfontein, southern Transvaal, overwintered inseminated foundresses of *B. petiolata* initiated nests singly. Joining by other females occurred at 53% of 81 nests to produce foundress associations containing 2 to 16 females, with a mean of 6.7 ($n=74$). Aggressive interactions between females produced linear dominance hierarchies, in which the most dominant foundress became the queen (primary egg-layer) and subordinate foundresses became non-reproductive auxiliaries.

Observations (277 hrs) on 22 colonies provided the following frequencies of behaviours for queens and subordinates, respectively: dominance acts, 0.91:0.09; oviposition, 0.97:0.03; oophagy, 0.98:0.02; larva-adult trophallaxis, 0.67:0.33; absence from nest, 0.11:0.89. Direct reproduction by subordinates was highly unlikely since eggs laid by these females were eaten by the queen in all observed cases.

Principal components and cluster analysis revealed substantial variability (continuous rather than bimodal) in body size and ovarian development among pre-nesting gynes ($n=91$); this probably formed the basis on which the dominance hierarchies were initially established. However, an increasingly distinct bimodality in ovarian development appeared among nesting foundresses ($n=277$, 86 nests) at successive stages of the colony cycle. All queens and 95% of single foundresses had well-developed ovaries as did 6 out of 9 replacement queens and several β -foundresses; all other subordinates had reduced ovaries. Queens were significantly larger with greater ovarian development than their β -foundresses ($p<0.005$, $n=20$, Wilcoxon matched-pairs). Since single foundresses had large ovaries, the hypothesis that single foundresses and subordinates have similar reproductive capacities (e.g. Dropkin and Gamboa, 1981) is not supported. The ability of subordinate foundresses to become replacement queens when the original queen was experimentally removed, demonstrated that ovarian regression was reversible.

Of an estimated 1716 gynes marked on their natal nests in autumn, 87.6% of 30 marked females recovered in spring, nested near their natal

sites. However, the probability of former nestmates reassociating at these sites was only 0.31; 15% of foundress associations consisted exclusively of former nestmates. Thus cofoundresses, on average, are not closely related.

Productivity (number of cells, larvae or pupae) and survivorship of multiple-foundress groups and therefore queens, increased significantly with number of foundresses per colony ($p < 0.05$; Spearman rho, $n = 32$ and X^2 contingency table, $n = 43$, respectively). Overall probability of multiple-foundress group survival was 0.42. Since single foundresses never produced pupae, their reproductivity was zero; no single foundress nests survived in any of 3 seasons (1982 - 1986).

The probability of foreign females evicting initial foundresses from their nests was 0.61 at single-foundress nests and 0.35 at multiple-foundress nests; the difference in the frequency of evictions at the two nest types was significant ($p < 0.05$, $n = 64$, X^2 contingency table). Joining females also had a 0.35 probability of usurping multiple foundress queens (i.e. when queens were not evicted but remained on nests as subordinates).

These results are interpreted as follows: (1) As single foundresses were incapable of producing colonies, joining behaviour is obligatory for survival and reproduction of foundresses, irrespective of individual reproductive capacity; (2) the low relatedness of cofoundresses and the high rate of initial foundress displacement by foreign females makes kin selection an unlikely explanation for foundress associations in this species; (3) the lack of a bimodality in body size or ovarian development among pre-nesting gynes indicates that queens are not manipulating their brood into queen-like and worker-like potential foundresses, and that parental manipulation is therefore of limited importance. Although the reasons for failure of single-foundress nests are unknown, we believe that adverse energetic factors, together with other environmental uncertainties (e.g. predation), preclude colony production by single females. We maintain that cooperative colony foundation evolved in response to strong directional selection derived from changed environmental conditions, which are probably still important in maintaining cooperative nesting in B. petiolata.

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Origin of Multiqueen Colonies Viewed from Observations of Tropical *Polistinae*

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The concept of inclusive fitness and kin-selection created a new epoch of theoretical studies on the evolution of eusociality in insects. Kin-selection theory predicts the relatedness between workers and new reproductives should be high: relatedness is high in monogynous colonies of honeybees, stingless bees and most vespine wasps, because the worker and new reproductive females are daughters of a single queen. Although there are many reported cases of polygynous foundation of colonies, it has been observed that polygynous colonies change to functionally monogynous ones based on a dominance hierarchy among cofoundresses (e. g. Pardi, 1948; West-Eberhard, 1969).

There are, however, many cases of the semi-permanent coexistence of many foundresses on a nest and even multiqueen colony system in the tropics.

SOCIAL BIOLOGY OF *ROPALIDIA FASCIATA*

Ropalidia fasciata lives in meadows in Okinawa. About half the nests are constructed by foundress groups (Table 1). I studied intranidal dominance acts among cofoundresses, colony survival rate and number of females with developed eggs in ovaries.

Intranidal relations among females of this species were significantly 'milder' than those of some species (e.g. *P. gallicus*, *P. canadensis*) for which 'severe' dominance relations are known (gothic numerals in Table 1 mean significant difference from *P. c.*). Many egg-layers coexisted on nests even after the emergence of progeny. Although *R. fasciata* sometimes showed typically aggressive relations, large variation in social behaviour is notable (C.V. of frequency of dominance acts/female/hr was about 100 % in *R. fasciata* and <40 % in *P. canadensis*).

A possible reason for this large flexibility is the significantly higher mortality rate of single-female colonies compared with multifemale colonies (Table 1,*), due to a high rate of ant predation, parasitism and destruction of nests by typhoons.

SOCIAL BIOLOGY OF SOME PANAMANIAN AND AUSTRALIAN WASPS

Table 1 also shows social relations and colony structure of some Panamanian and Australian species. It is notable that all species except *P. canadensis* showed no or a few dominance acts during the pre-emergence period, and there were multiple egg-layers on many nests of these species (except for *R. revolutionalis*). Although the sample size was small, it is notable that all the single-female colonies of 4 Neotropical wasps failed before emergence of progeny.

Table 1.(Numerals in parentheses mean sample size)

	% of multi- ♀nest	Survival rate of		% nests having		Freq.dominance acts/♀/hr	
		1♀-nest (till 1st emerg.)	♀♀-nest	PRE-E	POST-E	PRE-E	Post-E
Rf '83	53(58)	31(26)	* 64(28)	75(4)	56(9)	0.95±.95	0.12±.18
" '84	53(119)	36(55)	* 78(63)	-	-	1.06±.62	0.72±.50
" '85	64(47)	33(15)	* 88(25)	-	60(10)	0.32±.41	0.89±.79
Rr '84	80(10)	-	-	0(4)	-	0.06±.15	-
Rsp '84	83(6)	-	-	100(2)	33(3)	0	1.00±.68
Pg	-	-	-	-	-	1.95	4.43
Pc '82	98(90)	0(2)	* 50(12)	-	-	1.36±.55	2.61±.13
Pv '82	78(23)	0(4)	* 96(16)	50(4)	67(3)	0.09±.07	0.88±.81
Ma '82	82(11)	0(2)	*100(4)	100(3)	100(1)	0	#1.90±.44
Mb '82	61(18)	0(5)	* 75(4)	80(5)	100(1)	0.56±.22	#2.60±.13

Rf:*Ropalidia fasciata* (Okinawa), Rr:*R.revolutionalis* (Brisbane), Rsp:*R.sp.nr.variegata* (Darwin), Pg:*Polistes gallicus* (Italy), Pc:*P.canadensis* (Panama), Pv:*P.versicolor* (Panama), Ma:*Mischocyttarus angulatus* (Panama), Mb:*M. basimacula* (Panama). *:P<.001, Fisher EPT

Under such conditions where probability of successful colony foundation by a single foundress is nearly zero due to high predation pressure, multifemale foundation should evolve through individual selection (Lin and Michener, 1972). If large group size is necessary for colony survival, the dominant foundress should not attack subordinates so severely, because attacks may cause subordinates to leave the nest.

ESCALATION OF DOMINANCE BEHAVIOUR AFTER EMERGENCE OF PROGENY

In 2 *Mischocyttarus* species, the frequency of dominance acts during the post-emergence period was significantly higher than during the pre-emergence period (Table 1, #). A similar tendency is also seen in *Ropalidia* sp.(nr. *variegata*). I recorded the relative sizes of the attacker and the attacked individuals on post-emergence stage nests of 2 *Mischocyttarus* species. A large part of attacks (2/3) were made by larger females on smaller ones. I consider that the severe attacks seen on post-emergence nests of the 2 species indicates manipulation of progeny by mother (foundress) groups. If this is true, we can infer a possible route of evolution of multi-queen societies seen in Neotropical polybine wasps and some tropical *Ropalidia*. That is, evolution from (1) mutualistic permanent polygyny, through (2) manipulation of progeny by mother groups to (3) morphological differentiation of queens from workers.

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Eusociality and Parasitism in Nest-Provisioning Insects

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Eusociality has arisen repeatedly in the aculeate Hymenoptera and only once in other insect groups. This has led to a search for special characteristics of the Hymenoptera which might help explain how eusociality can evolve (Wilson, 1971). In order for this line of argument to be fruitful, it is important to keep in mind that aculeate evolution has proceeded in several different directions. Within the aculeates, eusocial species have been derived from ancestors which build and provision nests. Recent cladistic analyses by Brothers (1975) and Konigsman (1978) imply that the behavior of building and provisioning nests has arisen on at least 3 or 4 separate occasions in the aculeates. Within these nest-provisioning lineages, eusociality has arisen at least 12 times, but obligate cleptoparasitism has arisen at least 18 times. Obligate social parasitism, which is almost always derived from eusocial ancestors, has arisen many times (at least 65).

If a nest is defined as a structure which (1) provides shelter for developing young and (2) is provisioned with food carried into the shelter from outside by parents or helpers, such behavior has arisen in at least 11 taxa in 4 orders other than Hymenoptera, with major radiations in the termites, dung beetles, and fungus-growing scolytid and platypodid beetles. Although only one of these non-Hymenopteran lineages has given rise to eusocial species, parasitism is also quite rare in these groups. Social parasitism is unknown outside the Hymenoptera, and cleptoparasitism has only been reported in 3 species of dung beetles derived from nest-provisioning ancestors (Halffter and Edmonds, 1982). By comparison, cleptoparasitic aculeates derived from nest-provisioning ancestors have diversified into several thousand species in over 80 genera.

Theories about factors resulting in repeated origins of eusociality in the Hymenoptera should also take into account the multiple origins of parasitism in this group, relative to other groups having species which build and provision nests. In particular, this propensity for parasitism in aculeates has rarely been addressed in discussions of the importance of haplodiploidy and kin selection in the Hymenoptera.

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A Relationship between Paper Technology and Colony Size in the Social Vespidae

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Introduction. The Polistinae and Vespinae are highly social, but the maximum colony size of any stenogastrine species is about ten females. The absence of large colony size is consistent with the hypothesis that inability to construct a large nest has acted as a constraint on the evolution of large colonies in the subfamily.

Predictions of the hypothesis. Two of these have already found some support (Hansell, 1985). Here, two further predictions are examined: (1) that the fabric of the nest material of Polistinae and Vespinae will be superior to that of Stenogastrinae, (2) that the mandibles of Polistinae and Vespinae will be better adapted for chewing tough woody material than will those of Stenogastrinae.

Results. 1. Examination of the nest material of 8 species of Stenogastrinae, 16 of Polistinae and 3 of Vespinae showed that the material of Stenogastrinae is generally composed of short fragments of woody material held together by rather little salivary matrix. Some species include mud or use mud alone. By contrast, even small colony Polistinae tend to use long intact plant fibres and bind them with ample quantities of matrix. Some Polistinae do build large nests with apparently unsuitable materials like the massive nests of *Polybia singularis* made from mud. The nests of Vespinae, in spite of their large size, do not contain very long plant fibres.

2. The mandibles of Polistinae and Vespinae are shorter and broader (larger W/L ratio) (Fig. 1), and generally have a larger angle between them when the tips meet (Fig. 2) than those of Stenogastrinae. Both these features permit greater force to be exerted at the tip of the mandibles for a given muscle pull in the former two than the latter.

Conclusions. The predictions of the hypothesis are generally but not completely supported.

Reference. Hansell, M.H. 1985. The nest material of Stenogastrinae (Hymenoptera, Vespidae) and its effect on the evolution of social behaviour and nest design. *Actes. Coll. Insectes Soc.* 2, 57-63.

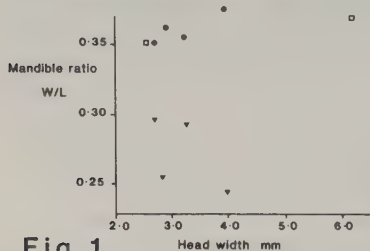


Fig. 1

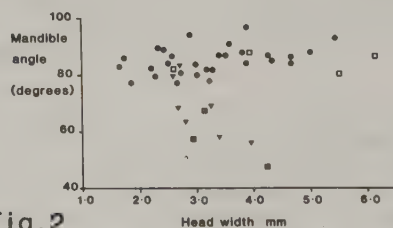


Fig. 2

Fig. legend. Solid circles = Polistinae; solid triangle = Stenogastrinae; open square = Vespinae; half solid square = Eumeninae

Size Disassortative Nesting Patterns within Aggregations of Solitary *Hymenoptera* - a Forerunner of Semisociality?

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Many species of solitary fossorial bees and digger wasps are known to nest gregariously, but little is known about the establishment and development of these aggregations or the selective forces operating in them (e.g. parasite and predator pressure). Associations between unrelated females nesting close together or sharing the same burrow has been suggested to be a possible early stage in the forming of social life in *Hymenoptera* (Lin and Michener, 1972, Alcock, 1975). Aggregations thus constitute an interesting field of research in the understanding of insect sociality and its evolution.

MATERIALS AND METHODS

The nesting behaviour in natural populations of *Colletes cunicularius* (Colletidae) and *Bembix rostrata* (Sphecidae) was monitored at the island of Öland (N 56 30; E 16 30), Sweden, in 1984, 1985 and 1986. Head and thorax width was recorded when the females emerged as were their nearest nesting neighbours for the first three weeks. Nearest neighbour distance was measured between burrow openings.

RESULTS

During the week of emergence female body size is distributed in a non random fashion. In *C. cunicularius* large females tend to emerge in the beginning of the period (spring), whereas in *B. rostrata* average body size is increasing (summer). Digging usually takes place within a few days, and both species exhibited nesting patterns with gradually increasing density within central parts of the nesting area. Due to the emergence pattern of different size groups, there was a difference in nest foundation date between these groups.

Average body size and nearest neighbour distance was found to differ between subsequent years. Neighbours were usually more dissimilar in body dimensions than was expected at random, indicating the occurrence of size disassortative nesting patterns within aggregations of this type. However, the pattern was fluctuating in character between years, associated with changing population densities. When density was high, small distances between nearest neighbours was associated with substantial differences in body size between these neighbours.

Individual females in *B. rostrata* displayed important behavioural differences within the nesting area. Such diffe-

rences were also observed between years, like the digging of "blind burrows" close to the nest entrances. This was also the case regarding "cleaning of the nest". In 1984 (high density) few females removed prey debris, leaving it outside the nest entrance. In 1985 and 1986 this behaviour was frequent, often attracting patrolling ants of several species (Larsson, 1986).

My data show at least two facts of significance:

- (1) Body size seemed to be related to the nesting pattern within aggregations of the species studied, and
- (2) major differences in behaviour and average body size can occur between generations.

The observed nesting pattern may partly depend on non random emergence of different size groups, but may also be related to other factors (Larsson, 1985, 1986). It is suggested that the occurrence of substantial differences in body size between nearest nesting neighbours may generate dominance relationships. Thus, it would represent a possible initial step on the semisocial pathway to sociality among Hymenoptera.

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Preadaptations to Sociality in the Solitary, Bivoltine Halictine Bee *Evylaeus villosulus* (K.)

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Morphological variation of this species has been described previously together with the nest architecture (Plateaux-Quénu et Plateaux, 1981, *Ann. Sc. Nat.*). It was then shown that spring foundresses and summer daughters were significantly different in size, colouration and sculpture and that the sex ratio of the summer generation was female biased (Plateaux-Quénu et Plateaux, 1984, *Act. Coll. Ins. Soc.*). That the summer daughters produce the larger overwintering females was proved in the laboratory (Plateaux-Quénu et Plateaux, 1986, *Act. Col. In. So.*).

Field work was performed at Jussieu, Paris, and bees were also reared in the laboratory. Field caught foundresses and summer females were compared using multivariate discriminant function analysis. The variables used were head width, wing length, two colouration and four sculpturing characteristics. Laboratory rearings confirmed the female bias of the summer brood. To calculate investment for sex ratios, pupal weights were used. Canonical discriminant analysis resulted in almost 80% of the 402 females (221 foundresses, 181 summer daughters) being separable on the basis of the 8 characteristics. Interestingly, the morphological variables did not show allometric variation within generations suggesting that the variation may perhaps be environmentally induced. The summer generation sex ratio was 3:1 females to males. The smaller weight of the male pupae converts this to an investment ratio of 5:1. The summer females were significantly smaller than their mothers and their daughters. Sample sizes for the autumn generation were rather small, but the investment ratio was also female biased (approximately 2.5:1). Unlike their sisters, summer and autumn males did not differ in size.

Investment ratios in both generations are female biased. This may result from local mate competition because the males from the same nest compete with each other for access to females in adjacent nests: summer males use to return to their natal nest after entering surrounding nests, probably to mate, although they also mate on flowers. These sex and investment ratios are in direct contradiction to those predicted by Seger's (1983, *Nature*) model which suggest that bivoltinism may produce a male biased summer generation and a female biased autumnal one and that this may promote the evolution of sociality by kin selecting for summer females that remain in the nest to raise sisters.

Of particular interest is the small size of the summer females and the female biased summer generation. Both of these characteristics being found in social species (Packer and Knerer, 1985, *Behav. Ecol. and Sociobiol.*), this may indicate preadaptations to sociality in this bivoltine solitary species.

FREE COMMUNICATION

Colony Compositions of the Subsocial Wood-Feeding Cockroaches *Salganea taiwanensis* Roth and *S. esakii* Roth (*Blattaria: Panesthiinae*)

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In discussions of the evolution of eusocial organization in Isoptera, the Blattaria is of special interest among the non-eusocial insects, because they are closely related to the Isoptera. Of the 4000 species of cockroaches in the world, a woodroach, *Cryptocercus* is believed to be the most primitive and the most closely related cockroach to the lower termites in phylogenetic relationships. On the other hand, some species of the Panesthiinae (Blaberidae) are also known to be wood feeding and wood-dwelling roaches. But considering the striking size and appearance of many different genera and species of the subfamily, it is surprising that very little is known of their biology. Therefore I studied the composition of colonies of the wood-feeding cockroach *Salganea taiwanensis* and *S. esakii* from summer 1983 to spring 1986 by field dissection of logs at the Nansei-archipelago in the southern part of Japan. Nine collection trips were made and approximately 50 social groups were sampled on each trip. The field evidence shows that the wood-feeding cockroaches live in the distinct monogamous family groups and the intimate adult pair-offspring relationships continue for a long time. The male-female pairs of adults, and the families consisting of a group of nymphs together with an adult pair, were the most frequently encountered colonies in the field, comprising 50.7% of the total social units (77.7% of total number of adults) in *S. taiwanensis* and 41.8% (66.4%) of the *S. esakii*. Overall mean brood sizes of family groups were 8.18 in *S. taiwanensis* and 7.40 in *S. esakii*. Single adults, single nymphs and groups of nymphs with no adult were 20.2%, 12.3% and 8.0% of the total social units, respectively, in *S. taiwanensis*, 22.6%, 8.9% and 8.9% of those in *S. esakii*.

Results of the field sampling reported here resemble those of *Cryptocercus punctulatus* studied by Seelinger & Seelinger (1983) and Nalepa (1982, 1984). The social organization of *Salganea* and *C. punctulatus* living in a family group for a long time are very atypical for cockroaches. Since the study MacKittrick (1964), it has been considered in many studies that *Cryptocercus* is a "primitive cockroach" and close to termites in phyletic relationship. However, like Deleporte (1985), I believe that the familial organization of *Cryptocercus* is more likely to be parallel to the evolution of termites, than phyletic relatedness making *Cryptocercus* a "primitive termite". This is because the social organization of *Salganea* living in a family group is very similar to that of *Cryptocercus*, although the phyletic relationship between *Salganea* and *Cryptocercus* in Blattaria is not regarded as close.

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4.1 Chemical Signals: Pheromones and Interspecific Interactions

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SYMPOSIA

Ant - Termite - Interactions

and

**Production Sites, Chemistry and Function of Exocrine
Secretions in Social Insects**

Organizers: Wittko Francke and Manfred Kaib

PLENARY LECTURE and Introduction:

Specificity of Pheromonal Signals: A Search for its Recognitive Bases in Terms of a Unified Chemisociality

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One of the major characteristics of eusocial species is the ability of nestmates to rapidly -- and accurately -- distinguish each other from alien individuals. In a sense, this great recognitive power was a sine qua non for the evolution of eusociality since it permitted nestmates to define colonial integrity in terms of a common olfactory nexus. Thus, the exclusiveness of the society was a function of an invisible badge of identity that was worn by all its members. However, while the existence of these societal badges has long been recognized, identification of their bases has proven to be for the most part, remarkably intractable.

For at least a century, biologists investigating social insects have both postulated and tried to prove the existence of colony odor, the common olfactory fingerprint that must be shared -- and recognized -- by members of a colony. This intranidal label has been attributed to intrinsic (genetic) and extrinsic (dietary) factors. However, although exogenously derived odorants may be of transient importance in contributing to colony odor, these organoleptic compounds are simply too unpredictable to qualify as the chemical bases for either specific or colonial nestmate recognition. It now appears likely that this pheromonal "holy grail" is not a product of exogenous odorants adsorbed onto the epicuticle, but rather, epicuticular compounds themselves.

Recognition of the cardinal significance of such semiochemicals has resulted in an exocrinological revolution--the confluence of diverse disciplines of exocrinology. This development is vividly illustrated by the topics presented in the symposia on "Chemical Signals." Morphologists, behaviorists, natural products chemists, evolutionists, toxinologists, biochemists, and ecologists have been drawn together as a natural consequence of their common interest in the exocrine glands--and their products--of social insects.

Diverse natural products, biosynthesized in complex exocrine glands, are demonstrated to regulate an incredible diversity of intra- and interspecific reactions. Glands have been adapted to subserve multiple functions as their often novel products are utilized as pheromones and/or allomones for friends and foes. The incredible potpourri of compounds synthesized

by the glands are becoming recognized as the cryptic behavioral triggers that made a viable eusociality possible. Although deciphering these pheromonal and allomonal languages has been difficult, it is becoming evident that the concerted efforts of sociobiologists with diverse specialties will ultimately create an exocrinological Rosetta Stone. It is implicit in such an eventuality that a hallmark of eusocial species--recognitive acuity--will become comprehensible as a key sociobiological element. Indeed, with such awareness, one can appropriately view an insect colony as the culmination of an evolutionary scenario in which chemisociality has been an omnipresent driving force.

In the brief presentation that follows, I will develop the thesis that colony odor is first and foremost, a cuticular pheromone that appears to be identified with the epicuticular hydrocarbons. I will also focus on the adaptiveness of this hydrocarbon layer as a surface pheromone, and emphasize its compositional idiosyncrasy as a key characteristic that made it suitable as an easily recognizable colonial signature. In addition, I will analogize this hydrocarbon layer to a gas-chromatographic stationary phase vis-a-vis both endogenous (pheromones) and exogenous (dietary) volatiles. Finally, I will stress the genetic control of the biosynthesis of quantitatively distinct blends of hydrocarbons as an important factor in kin recognition.

EPICUTICULAR HYDROCARBONS AS RECOGNITIVE SURFACE PHEROMONES

Part of the epicuticle consists of lipids which are often dominated by an extensive series of aliphatic hydrocarbons belonging to several classes. A large number of n-alkanes, n-alkenes, monomethylalkanes, dimethylalkanes, and trimethylalkanes have been identified as cuticular constituents (Blomquist and Dillwith, 1985), and closely related species have not been reported to possess the same hydrocarbon profiles (Howard and Blomquist, 1982). In addition, each species produces blends of hydrocarbons that are often qualitatively, and invariably quantitatively, distinct (Blomquist and Dillwith, 1985). If insects were able to perceive the epicuticular hydrocarbons of conspecifics with discriminatory precision, this surface pheromone would constitute a functional species-specific badge. Since it covers the entire surface of the insect, this cuticular pheromone could be readily perceived when any body region is antennated by a conspecific. Furthermore, the long-chain hydrocarbons of the epicuticle would possess a low enough vapor pressure to produce a small active space and thus qualify as a surface pheromone that could be detected either by contact or at very close range.

Identifying the cuticular hydrocarbons as a pheromonal signal simultaneously classifies their

derivative epidermal cells as exocrine structures capable of synthesizing very distinctive cuticular constituents. In particular, the well-developed ability of eusocial species to biosynthesize methyl-branched alkanes from succinate or propionate via methylmalonyl CoA (Chu and Blomquist, 1980) has enabled these arthropods to produce blends of hydrocarbons which contain remarkably idiosyncratic compounds. Cuticular hydrocarbons endowed with different proportions of these unusual branched alkanes should constitute highly distinctive olfactory signals enabling "tuned" species to rapidly discriminate nestmates from aliens. In a real sense the specificity is the blend (Blum, 1974).

Long-chain alkenes and methyl-branched alkanes function as contact sex pheromones for dipterans, so their utilization by eusocial species as contact pheromones would be neither novel nor surprising. Indeed, it will prove surprising if cuticular hydrocarbons (=surface pheromones) are not utilized widely for species recognition throughout the Arthropoda.

Intercolonial differences in cuticular hydrocarbons have been easily detected by utilizing pattern recognition procedures (Brill *et al.*, 1986). Furthermore, computer-based pattern recognition procedures can be successfully utilized to distinguish the hydrocarbon profiles of individual workers from the same colony. However, notwithstanding the fact that species-, colony-, and caste-specific patterns characterize the cuticular hydrocarbons of eusocial insects, the question arises whether these cuticular profiles can be demonstrated to be of behavioral significance as recognition pheromones. Current evidence suggests that the answer is yes.

BEHAVIORAL SIGNIFICANCE OF CUTICULAR HYDROCARBONS

Confirmation of the cognitive importance of cuticular pheromones has been difficult to obtain because of inherent bioassay problems. Nevertheless, the results of several recent studies suggest that cuticular hydrocarbons are key integrative agents.

Howard *et al.* (1980) have demonstrated that the highly integrated termitophile Trichopsenius frosti synthesizes the same cuticular hydrocarbons as its host, Reticulitermes flavipes, and this congruence in hydrocarbons appears to play a key role in the integration of this beetle into termite societies. It has also been demonstrated that another myrmecophilous beetle, Myrmecaphodius excavaticollis, tactually acquires the cuticular hydrocarbons of its host, thus ensuring that its recognition is infallible (Vandeer Meer and Wojcik, 1982). Beetles rapidly lose their counterfeit cuticular hydrocarbons if removed from the host's nest but can rapidly acquire different hydrocarbons if placed with a new host species.

The implications of these results vis-a-vis parasitic ants are obvious and exciting. I predict that these parasitic formicids, which are closely related to their host species, will prove to possess surface pheromones, and others, that are both quantitatively and qualitatively similar to those of their hosts. In effect, these parasitic ants would be masking their alien status with the same hydrocarbon "clothes" as their hosts.

Crucial behavioral studies which are consistent with the role of cuticular hydrocarbons as colony recognition agents have been undertaken recently by Bonavita-Courgourdan *et al.* (1986) with the very aggressive formicine Camponotus vagus. Workers from a single colony produce characteristic proportions of dimethylalkanes that appear to constitute a distinctive intranidal fingerprint. When the isolated cuticular hydrocarbons from workers in one colony are applied to dummies placed in a different colony, strong aggressive behavior is elicited. Workers do not exhibit agonistic behavior when they are presented with dummies treated with hydrocarbons derived from workers in their own colony.

CUTICULAR HYDROCARBONS AS A GAS-CHROMATOGRAPHIC STATIONARY PHASE

Although it has been frequently suggested that both exogenous volatiles and endogenously derived pheromones could be absorbed in the epicuticle, there is no reason to believe that this absorption is a passive process. Rather, it is not unlikely that the cuticular hydrocarbons could be functioning as the equivalent of a gas-chromatographic stationary phase. Such a function would be consistent with the role of these hydrocarbons as versatile surface pheromones. These hydrocarbons could function as a virtual capillary layer by fractionating volatiles that are absorbed in them, if the insect is regarded as a relatively uniform heat source (=gas chromatograph).

For example, Butler *et al.* (1974) have demonstrated that tritiated queen substance, 9-oxo-(E)-2-decenoic acid, is rapidly translocated to the abdomen of worker honey bees after topical application to the thorax. There is no reason why other pheromones, secreted from either cephalic or abdominal glands, could not be rapidly distributed by translocation on the body. Indeed, the specific identities of eusocial species should be made more absolute by distributing pheromones this way.

CUTICULAR HYDROCARBONS AND KIN RECOGNITION

One of the obvious correlates of producing specific proportions of cuticular hydrocarbons is that these blends should be more quantitatively similar in closely

related individuals. Thus, queens should produce daughters with similar biosynthetic capabilities for particular cuticular hydrocarbons and kin evaluations could thus be based on quantitative olfactory assessments. Ultimately, it may be possible to demonstrate that tight family bonds are actually the carbon-to-carbon bonds in the magical hydrocarbons that insect societies utilize as their idiosyncratic family badges.

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Frontal Gland Secretion and Colony Defence in Termites: Evolutionary Aspects

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Modern termite species live in eusocially organized colonies with large numbers of individuals concentrated in monocalic or polycalic nests. Because of the locally high concentration of protein in their hives, termites are an excellent food source to predators. The large numbers of predators cause a considerable predation pressure on the termites which is believed to be the most important biotic barrier confining the distribution of termites (EMMERSON, A.E. 1955: *Fieldiana Zool.* 37, 465-521). For the adaptive evolution of termite defense strategies predation by ants is most important for two reasons. Firstly, there is a constant predator-prey interaction between ants and termites - in contrast to occasional or seasonal predation by e.g. vertebrates. Secondly, ants have well-developed and efficient recruitment systems which enables them to utilize food sources rapidly.

To counteract ant predation, termites have evolved a considerable variety of defense strategies on a communal level as well as on an individual level. On a communal level termites protect themselves from attacks by physical separation from the ants e.g. by their nest structure or by covering foraging galleries or foraging sites. During individual combat defense may be organized at a communal level as well: soldiers and in some species workers are recruited to the sites of combat and then counteract ant attacks by individual fighting.

With few exceptions all termite species have developed a soldier caste which, in general, exhibits extreme morphological specializations (form and size of mandibles) towards defense. Mandibular defense is common throughout the Isoptera. However, considering the much smaller size of most termite species, compared with predatory ants, the effectiveness of mechanical weaponry appears to be rather limited. Thus additional defense strategies have been developed.

Only two termite families - Rhinotermitidae and Termitidae - have developed a frontal gland the secretion of which is used in some species for defense. The use of chemical defense can be nicely demonstrated in the termite subfamily Nasutitermitinae. Through a conical nose their soldiers spray a sticky fluid onto predatory ants and entangle the predators without any physical contact (ERNST, E. 1959: *Rev. Suisse de Zool.* 66, 286-295, EISNER, T. et al. 1976: *Behav. Ecol. Sociobiol.* 1, 83-125). Soldiers of the lower termite *Schedorhinotermes* (Rhinotermitinae) also use frontal gland secretion (FGs) for the colony defense, however, the defense strategy is based on different mechanisms. During combat with ants FGs is released and flows into a terminal brush at the soldier's labrum. There it is kept by capillary forces and rapidly evaporates due to the extreme extension of the surface. This secretion is not sticky nor toxic, but volatile constituents (Vinylketones and Dienones) repel ants and inhibit the ant recruitment mechanisms (KAIB, M. 1985: *Mitt. dtsh. Ges. allg. angew. Ent.* 4, 302-306). In *Myrmecaria eumenoides*, an important termite predator (LEVIEUX J. 1983: *Insectes Soc.* 30, 165-176), both, short range and long range recruitment is interrupted by FGs (DITEBRAND and KAIB, this volume). Thus mass attacks by ants do not occur.

The efficiency of the inhibition of the ants' recruitment is even increased by the polyethism during foraging in *S. lamanianus*. Scouting outside the protective galleries is only done by minor soldiers which then lead termite workers to newly discovered food or water. Although recruitment is initiated by termite workers (SCHEDEL and KAIB, this volume), they are always guarded by minor soldiers thus building up a defensive barrier against ants.

The interruption of recruitment occurs not only in *M. eumenoides* but also in all ant species investigated so far which live syntopically with *S. lamanianus*, and which belong to all the major groups in the Formicidae. It also occurs in ant species from North African arid areas and from Europe. This means that the anti-recruitment function of the frontal gland secretion of *S. lamanianus* is effective even in modern ant species which never encounter *S. lamanianus*. Thus by emitting ketones from the FGS into the air, this termite possesses a generally effective defense strategy against ant predation without physical contact with the predator.

The generally effective defense by FGS of *S. lamanianus* raises two major questions: 1) Why did ants not succeed in penetrating the defensive barrier? 2) How and when did this defense strategy evolve? Although ants show extreme ecological adaptations and food specializations they apparently were not able to penetrate this defense barrier. An answer to this question may be given by comparing the effectiveness of the FGS of termite species syntopic with *S. lamanianus* and with *M. eumenoides*. Applying a sticky secretion onto predators (e.g. as in *Coptotermes* which produces only few soldiers but has a huge frontal gland reservoir) small ants may be disabled. This mostly causes the loss of the soldier, and it does not protect the termite colony from mass attacks.

With the exception of *Amitermes unidentatus* which also produces ketones in the FGS of the soldiers, no African termite other than *Schedorhinotermes* inhibits ant recruitment by volatile constituents at the FGS and thus does not protect from mass predation. It appears that *S. lamanianus* can maintain its dispersed nest structures and its extended arboreal foraging in the open because of its efficient chemical defense and because of the presence of other syntopic termite species relying on mechanical defense. These species have to confine their activities to underground or to ranges limited to areas sheeted with soil particles.

When did the general defense strategies of *Schedorhinotermes* evolve? This question cannot be clearly answered. The genus *Schedorhinotermes* is of Indomalayan origin and reached the Australian region via the Papuan in early Cretaceous times. The westward movement to the Ethiopian region seems to have occurred in the Eocene or even later. A comparison of the chemical composition of the FGS in *S. lamanianus* with that in *S. putorius* in West Africa (QUENNEDEY, A. et al. 1973: Insect Biochem. 3, 67-74), in *S. intermedius* from Australia and in *Schedorhinotermes* from Malaysia - which has not yet been identified to species - shows very similar profiles with the main components being identical. Ketones appear to be a general characteristics of the genus *Schedorhinotermes* and thus may have been constituents of their FGS already in early Cretaceous times.

The oldest ant known lived in the middle of the Cretaceous period (WILSON, E.O. 1971: "The Insect Societies", Belknap Press, Cambridge, Mass.). This means the genus *Schedorhinotermes* possessed the FGS at or even before the time the ants started their development. Thus ant predation has been no evolutionary primer for *Schedorhinotermes* to develop its FGS for chemical defense. It originally must have had a different function than defense. Vinylketones are products of the metabolic fatty acid pathway and occur in all animals, but at different concentrations. In *S. lamanianus* FGS also plays a role in caste determination (RENOUX, J. 1976: Insectes Soc. 23, 279-474) which may apply to different termite genera as well. In most termite genera the proportion of soldiers to workers is very low (1 to 5%) whereas in others like in Nasutitermitinae (DELIGNE, J. et al. 1981: in: "Social Insects" (ed.: R.H. Hermann) Vol II, Academic Press, New York) or in *S. lamanianus* (KAJB 1985) the proportion of soldiers is very high (up to 40%). Termite species having high soldier proportions rely on chemical defense, which enables them to forage in the open and to live in dispersed colonies. Termite species with low soldier proportions use other defense strategies than based on the FGS. In these species the FGS appears to have different functions than defense. They live cryptically and in spatially confined nest areas.

TLC (silica gel, 6:3:1::iPrOH:conc. $\text{NH}_4\text{OH}:\text{H}_2\text{O}$).

RESULTS AND DISCUSSION

In a typical *in vitro* experiment, 25 soldiers were cooled to 4° C, decapitated, and heads homogenized as described above to give 0.5 ml of supernatant. Substrate (3,000-4,000 dpm) is added to a 0.2-ml aliquot of supernatant and incubated at 30° C for 4 hr. The solution is extracted with three 0.2-ml aliquots of 20% ethyl acetate in hexane and the organic layers are dried (MgSO_4), concentrated to 20 μl , and spotted onto a Whatman LK 20 x 20 cm TLC plate and eluted with 20% ethyl acetate-hexane. Plates were then scanned for radioactivity in the regions corresponding to the diterpene hydrocarbons, mono-alcohols, and diols (Bioscan System 500 Linear Analyzer).

Preliminary results indicate that, like our cembrene-A experiments, geranylgeranyl pyrophosphate is not measurably converted to defense secretion diterpenes with these methods. High levels of pyrophosphatases appear to be present in the tissue extracts, leading to a predominance of labeled geranylgeraniol as a neutral product. In contrast to the straightforward results for the incorporation of acetate and mevalonate into the defense secretion, it appears that the biosynthetic enzymes do not get access to advanced diterpene precursors administered in these simple experiments.

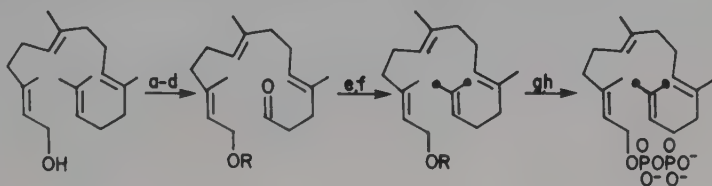


Fig. 2. Synthesis of [^{14}C]-GGPP

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Isolation and Identification of the Defensive Compounds of the Termite Species *Nasutitermes nigriceps*

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Nasutitermes nigriceps is a highly evolved termite species which uses exclusively chemical means for defense. The composition of the soldier's frontal gland secretion has recently been investigated.

The soldiers of termites for our studies were collected in Peru, Iquitos locality. The secretion was extracted by ethanol. The extract was separated in two crude fractions according to the volatility. The volatile fraction was identified as a mixture of monoterpene and sesquiterpene hydrocarbons, which was analyzed by GC-MS. Six monoterpenes (α - and β -pinene, camphene, myrcene, limonene and terpinolene) and two sesquiterpenes (cis-caryophyllene and γ -gurjunene) have been identified by comparison of their mass spectra with the known data.

In the non-volatile fraction of the extract we could identify five diterpenic alcohols (I-III, VII, VIII). Four of them were derivatives of the tricyclic trinervitane, while the remaining one had the tetracyclic rippertane skeleton. The compounds I-III and VIII were found earlier in other termite species. Their identity was verified by comparison of their mass, IR and NMR spectra with those of authentic samples. An extensive literature search revealed that the alcohol VII had NMR spectra identical with those of an alcohol isolated by Prestwich in 1978 from *Irinervitermes graciosus* soldiers, for which he proposed structure VI (Prestwich, 1978). The identity was verified by direct comparison with an authentic sample kindly provided by Prof. Prestwich. However a detailed analysis of NMR spectra of this compound and its derivatives led us to the revision of this structure.

The position of the hydroxy group in alcohol VII have been determined on the basis of decoupling experiments. By irradiation of the CH-OH proton (δ 3.93) we could assign the signals of neighbouring methylene protons (δ 2.34 and 1.79). For one of these methylene protons (δ 2.34) we have found also the homoallylic coupling with C(15)-methyl. Therefore the structural fragment -CH(OH)-CH₂-C=C-CH₃ was proved and hydroxy group localized into position 3. The Prestwich's structure VI with hydroxyl in position 2 does not agree with our decoupling experiments.

Further we had to solve the question of the configuration of the hydroxy group in position 3. For solution of this problem we decided to use the diastereoisomeric esters of alcohol VII with (R)- resp. (S)- α -methoxy- α -(trifluoromethyl)phenylacetic acid. Both esters were prepared from alcohol VII and the corresponding acid with 2-chloro-1-meth-

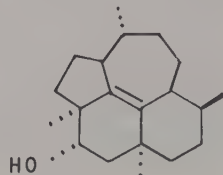
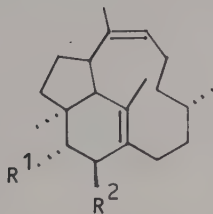
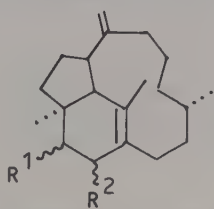
ylpyridinium iodide as the condensation agent in the presence of dimethylaminopyridine as a base. It is well known from the literature (Rinaldi, 1982) that acidic part of these esters prefers the conformation with the planar "zig-zag" arrangement of bonds CH-O-CO-C-CF_3 . The NMR determination of the absolute configuration then makes use of the different predictable shielding effects of the phenyl ring on the protons in the neighbourhood of the esterified hydroxyl - in our case C(4)-methyl and C(2)-methylene protons. From the experimental data follows the configuration $3\alpha\text{-OH}$.

In order to establish the intraspecific variations in the composition of the defense secretion, we have studied termites from three different nests in Peru. Recently, Prof. Prestwich and his group has published the results of the analysis of the defense secretion of the same species collected in Costa Rica (Gush et al., 1985). Thus, we can compare two allopatric populations of this species. In the volatile fraction, the basic difference is in the presence of sesquiterpenes in our Peruvian colonies. In the non-volatile fraction, Gush found two more trinervitane derivatives IV, V, which were not present in our colonies. Hence, we can conclude that the composition of the defense secretion is not strictly species-specific, but rather depends on the place of occurrence.

In order to assess the biological activity of the defense compounds, we tested the toxicity of three monoterpenes and two diterpenes with three potentially conflict insect species. The most toxic compound was alcohol II which LD_{50} was by 1-2 orders of magnitude lower than in the case of all other compounds tested. A simultaneous application of a monoterpene (limonene) with a diterpene (diol II) aimed at checking a possible synergism, gave negative results.

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- I, $R^1 = \alpha\text{-OH}$, $R^2 = \alpha\text{-OH}$
 II, $R^1 = \beta\text{-OH}$, $R^2 = \alpha\text{-OH}$
 III, $R^1 = \alpha\text{-OH}$, $R^2 = \text{H}$
 IV, $R^1 = \alpha\text{-OH}$, $R^2 = \beta\text{-OH}$
 V, $R^1 = \alpha\text{-OH}$, $R^2 = \text{O}$

- VI, $R^1 = \text{H}$, $R^2 = \text{OH}$
 VII, $R^1 = \text{OH}$, $R^2 = \text{H}$

VIII

Geographic Variation of Nasute Termites in Savanna "Islands" of Venezuela

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Chemical, morphological, and biochemical variation among nasute termites is being used to test the Pleistocene Refugia Hypothesis. This hypothesis - a possible explanation of the high species diversity of organisms in the Amazon Basin - asserts that isolated patches of rainforests served as foci of speciation when the Amazon Basin was an extensive savanna. Today, this area is covered by tropical rainforest, with isolated patches of savanna vegetation scattered throughout the region. Plant geographers feel that these "islands", found deep in the rainforest regions of the Amazon, are relicts from the Pleistocene savannas of 10,000 years ago.

The termite species that inhabit these "islands" are characteristic of savannas, and are restricted to grasslands. The termite populations which occur in the "islands" are isolated, often by hundreds of miles, from congeneric populations on other "islands" or the "mainland" llanos. Because of this isolation, each of the populations is subject to independent selection, which could result in the evolution of separate species. Thus, these populations are modern day analogs to those living in the rainforest refugia during the Pleistocene.

The data reported here are the preliminary results of a study on two of these populations - from La Esmeralda and Culebra. La Esmeralda is the most southern savanna "island" in Venezuela. It is an extensive and fairly complex savanna covering more than 10 km². In contrast, Culebra is very small (less than 1 km²) and homogeneous. These two sites are less than 40 miles apart yet are completely isolated from each other by surrounding rainforest and the western face of a 2250 m high tepui. The nearest margin of the llanos is 250 miles to the northwest.

As can be seen in the data, variation in defensive chemical, biochemical, and morphological characters is much greater at La Esmeralda than it is at Culebra. If the climate were to change again, these populations could form distinct groups and possibly even species.

My appreciation to Glenn Prestwich, Maureen Dunn, Barbara Thorne, Hernan Romero, and Philip Stansly for assistance in the field.

Geographical Variations in Termite Defensive Compounds and Efficiency against their Predators

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European termites of the genus *Reticulitermes* represent a fantastic feeding resource for many carnivorous insects. Their major predators are ants, especially *Myrmicinae*. *Reticulitermes* soldiers synthesize defensive compounds to protect the nest in different amounts according to their geographical origins. These compounds are monoterpenes = pinene, limonene; sesquiterpene = germacrene A and one diterpene alcohol = geranyl-linalool. Toxicities of these terpenes alone or mixed were tested on various predators. The main compound = the geranyl linalool is also the most efficient; better for some predators than commercial insecticide. Quantitative geographical variations between termite populations are well correlated with various predator resistances.

Chemical Mimicry of *Hypoponera eduardi*, Predatory Ant of European Termites

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Hypoponera eduardi is a predatory ant for european termites of the genus *Reticulitermes*. Ethological analyses showed that this ant is not recognised as an alien by workers and soldiers in a termite nest. On the other hand, ant workers living in a nest of an other termite species, introduced in the nest are detected and killed. A fine ethological analysis indicated that termites are able to discriminate an ant's origin during contact stimulations. Chemical analyses (GC/MS) of cuticular hydrocarbons of non-aggressive termites and *Hypoponera* showed that some major compounds are similar (especially monomethylalkanes with 25, 27 and 29 carbons branched in carbon number 11 and 13).

Food Recruitment in the Ant *Myrmicaria eumenoides*, Influenced by the Defense of *Schedorhinotermes lamanianus*

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A well developed food recruitment system enables the common African ant *Myrmicaria eumenoides* to prey effectively on termites, which represent their main protein source. Having discovered a rich food source (e.g. a termite colony) scout ants quickly return to their nest dragging the abdominal tip over the ground and depositing scent marks. This behaviour is initiated by contact with food and occurs in >85% of ants returning from the food to the nest. Also, scout ants in combat with prey release chemical signals by extruding the sting and depositing sting-apparatus secretion (SAs) onto the ground or onto the prey. Thus additional ants are recruited from nearby foraging areas towards the sites of combat to overwhelm the prey jointly. This leads up to a tenfold concentration of ants at the prey (short range recruitment).

The SAS is composed of a volatile (1/3 of the total secretion) and a non-volatile (2/3) fraction. So far there is no evidence that the non-volatile fraction functions in orientation and recruitment behaviour. The volatile fraction contains hexanenitrile and six monoterpene hydrocarbons among which limonene is the main component (97%).

Ants respond equally to synthetic limonene and to SAS. Limonene effects dose dependent recruitment of ants from short range to the prey. The application of 0.5 μg of limonene onto the prey increases the aggregation of ants by 120%.

In laboratory colonies, the vapour of SAS applied into the nest does not recruit ants towards the foraging arena. This means that long range recruitment is not triggered by SAS or limonene on its own. However, it is effective when applied in combination with mechanical stimulation (antennal invitation behaviour) by scout ants returning from food. Due to an age dependent polyethism of *M. eumenoides* approx. 1/3 of 400 take part in foraging. A single successful scout ant is able to recruit 60 ± 10 ($n=10$) nestmates out of the nest within a period of 2 minutes.

One of the strategies of the termite *Schedorhinotermes lamanianus* counteracting ant predation is the use of frontal gland secretion. Under natural conditions of combat this secretion is not toxic to ants (KAIB, 1982: Verh. Dtsch. Zool. Ges., 315), but it inhibits both short range and long range recruitment of the ants.

Following localisation by the ant 67% of the termite workers are attacked and a total of 37% are transported to the nest. Those workers lost after attack may be recaptured due to the efficient short range recruitment of the ants. When only minor soldiers are present, the incidence of attack by ants is reduced to less than 1/3. Equal numbers of attacks are initiated by ants and minor soldiers which are more agile and aggressive than workers. The vapour of FGS released by soldiers during former attacks repels ants preventing physical contact with the predators. Soldiers escaping or lost are not recaptured because of the inhibition of short range recruitment of the ants. Less than 1% of initial encounters lead to transport to the nest. Since termite soldiers are not transported to the nest, long range recruitment is not initiated. Ants returning to the nest from encounters with soldiers do not deposit scent marks even if they had previous contact with termite workers. Thus long range recruitment is interrupted which persists for at least 20 minutes. This gives the termites sufficient time to retreat into the galleries and close the exits, separating predators and prey.

Salivary Gland Morphology Related to Polyethism in the Termite *Macrotermes bellicosus*

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The salivary gland in termites is known to play a role in digestion, food transfer, building behaviour and colony defence (Grassé, 1982). The aim of this contribution was to describe the morphology and ultrastructure of the salivary gland in *Macrotermes bellicosus*, and to find out whether the gland shows any morphological peculiarities between the different castes and polyethic groups.

As in other termites (Pasteels, 1965; Grassé, 1982), the salivary gland in *M. bellicosus* is composed of numerous spherical acini, from which small ducts lead to the main efferent duct. According to the appearance and size of cytoplasmic vesicles, 3 secretory cell types can be discerned in the acinar lobes of the workers. The most prominent are types I and II, in which the greater part of the cell volume is occupied by electron-lucid vesicles (all equally small in type II, with distinctive variation in size in type I). The small and peripheral type III cells are characterized by their extensive apical cell border that abuts onto the more centrally located ductule cells. Their cytoplasm contains numerous mitochondria and electron-dense secretory granules near to the microvilli in adult workers, which are lacking in the larval stages. The general features of the salivary gland in *Macrotermes* correspond to the earlier description in cockroaches (Day, 1951). However, in addition to the prominent secretory apparatus of the type III cells, cell types I and II also display a microvillar contact area with the ductule cells, which indicates their independent discharge of secretion in *Macrotermes*.

In the soldiers the acini are composed of only one type of secretory cell that is characterized by a well developed microvillar region near the centre of the acini, and mainly small electron-lucid vesicles.

Ultrastructural differences do not occur among the adult worker groups. The general development of the salivary gland, as measured from the acinar size, however, varies according to castes and polyethic groups. It considerably increases between the last larval and the final adult stages, and attains a maximum size in the functional class of nursing workers. The relative size in minor workers, however, is larger than in the majors. In older workers, that are involved in foraging and building behaviour, the gland is reduced but not degenerated, indicating that it is still functional. The smallest acini are found in the soldiers.

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Polyethism During Foraging in *Schedorhinotermes lamanianus* in Unprotected Areas: the Role of Exocrine Glands

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S. lamanianus has workers and dimorphic soldiers all possessing well developed sternal glands for trail laying. The colonies possess a widely spread foraging area, with galleries leading from the nest to the foraging sites. Workers and minor soldiers (MS) take part in foraging.

Results

1.) Foraging behaviour was observed under laboratory conditions. During foraging two different phases can be observed: a) initial exploration, b) recruitment. MS initiate exploration. It is characterized by: individual searching behaviour of MSS, small numbers of individuals of both castes outside the gallery, more soldiers than workers being present. Recruitment starts when the first worker scout returns to the gallery after discovering food. On its way back it lays a recruitment trail, which leads nestmates to the food source. Recruitment is initiated by workers. It is characterized by: recruitment of nestmates - workers and soldiers -, orientation along the recruitment trail, more workers than soldiers being present outside the gallery (worker/soldier ratio: 2:1). Both castes show trail laying behaviour during exploration and recruitment.

2.) Thresholds of MSS and workers for similar extracts were determined with standard methods. MS respond more sensitively to sternal gland extracts from both castes (worker/soldier ratio a) worker extract: 1: 0.63 \pm 0.02; b) MS sternal gland extract: 1:0.61 \pm 0.07).

3.) Trail pheromone-concentration was determined by behavioural tests, frontal gland secretion (FGS) by gc-analysis. Worker extracts were contaminated with defined volumes of FGS. On trails with high pheromone concentration following is not influenced by FGS. However, on pheromone trails with concentration just above threshold there is dose dependant inhibition by FGS in both workers and soldiers.

Conclusion

Foraging is initiated by minor soldiers. During exploration only trails being reinforced by frequent commuting are followed by termite workers. Minor soldiers are not able to elicit mass recruitment, however there is an orientation effect of the SGS of MS. This is similar to *Nasutitermes costalis* (TRANIELLO, BUSH, 1986, J. Chem. Ecol. 11: 319-332), where trail pheromone extracts of soldiers are not able to recruit workers.

In *S. lamanianus* mass recruitment is initiated only by one (successful) worker. It lays a recruitment trail, which is very attractive to nestmates (probably because the pheromone concentration is high).

The different caste response during exploration is based on the different pheromone sensitivity of workers and MSS. MSS operate further away from the gallery end, where the pheromone concentration is lower. Thus intruding ants are confronted first by MS, which release FGS during com-bat. Vapour of FGS reduces the trail pheromone sensitivity of both castes. This prevents workers from leaving the gallery, whereas MSS remain out-side. All this together limits the activity of workers to the area close to the gallery exits, especially during predation.

Novel Pyrrolizidine Alkaloids from Myrmecine Ants Native to New Zealand

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Myrmecine ants of the genera Solenopsis and Monomorium are known to utilize an array of alkaloids, which are major components of their venoms, for predation and defense. Most commonly, the saturated nitrogen heterocycles found in these venoms are 2,6-dialkylpiperidines and 2,5-dialkylpyrrolidines. Occasionally, 3,5-dialkylindolizidines and rarely, 3,5-dialkylpyrrolizidines have also been found (Jones, et al., 1982; 1984). The ants produce these compounds with remarkable stereoselectivity. With only one exception, the monocyclic alkaloids have been found predominantly as the trans isomers in Monomorium and Solenopsis species. Furthermore, the 3,5-dialkylindolizidines produced by both genera are the (5Z,9Z) stereoisomer.

We have recently had the opportunity to examine the extracts of numerous collections of ants in the genus Chelaner obtained in New Zealand. The alkaloids that predominate in these extracts are 3,5-dialkylpyrrolizidines, and we have established the structure and stereochemistry of two novel pyrrolizidines by independent synthesis.

MATERIALS AND METHODS

Single-nest collections of the ants were made by Prof. A.W. Don, Zoology Department, University of Otago, Dunedin, New Zealand, and stored in vials containing methylene chloride. The structure determinations followed previously described methodology wherein the pyrrolizidines, suggested by GC/MS examination of the methylene chloride extracts, were synthesized by reductive amination of the appropriate triketone (Jones et al., 1980; 1986).

RESULTS

The predominant alkaloid found in twenty-two of the thirty-two collections of ants was (5E,8Z)-3-(1-non-8-enyl)-5-(E)-1-prop-1-enylpyrrolizidine(I) (Jones, et al., 1986). In ten of the collections, the major alkaloidal component was (5E,8Z)-3,5-di(1-hex-5-enyl)pyrrolizidine (II), and in a single collection, the major alkaloidal component was (5Z,8E)-3-methyl-5-(1-non-8-enyl)pyrrolizidine (III). Although pyrrolizidines I and II are isomeric, they are not found together in these ants, whereas pyrrolizidine III occurs together with traces of I. In addition, the presence of traces of 3-allyl-5-(1-non-8-enyl)pyrrolizidine and 3-butyl-5-(1-hex-5-enyl)pyrrolizidine as minor components is strongly suggested by mass spectra and by the presence of concomitant pyrrolidines. Since these alkaloids are detectable only in trace amounts, their structures remain unconfirmed.

European *Monomorium* Alkaloids: Toxicity Against Preys and Emitters

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In western Europe, termite societies in the genus *Reticulitermes* suffer predation from many ant species. Ants use different strategies of aggression. In the genus *Monomorium*, a particular chemical weapon is used: the venom is not injected, but only deposited on the prey's cuticle. This venom contains toxins able to paralyse and kill the preys. The chemical nature of the toxins was previously described (1), and the toxicity of each constituent has been measured separately, using a toxicity bioassay.

MATERIAL AND METHODS

Ants were collected in Saint-Trojan (Charente-Maritime), close to a termite society of *Reticulitermes*. The chemical analysis was performed with a GC/MS coupling technique.

In the bioassay, a range of dilutions was prepared, using acetone as a solvent. 5 batches of 10 *Reticulitermes* workers received each 1 μ l of the solutions. Deaths are counted 15 h. after, and the LD50 is calculated in μ g/mg of termite, by the probit method. The ants received 0.5 μ l of the solutions, following the same method.

RESULTS

The venom is a mixture of alkaloids: 3 dialkylpyrrolidines (Pyr1,2,3) and 2 dialkylpyrrolines (Pyr4,5). These pyrrolidines and pyrrolines are common among the genus *Monomorium* and *Solenopsis* (2,3), though one (2(1hexyl-5enyl-5nonyl)pyrroline: Pyr5) is a novel alkaloid.

4 compounds, obtained from synthesis in the chemistry laboratory or isolated from the ants, have been tested in the bioassay. They all show an important toxic activity against *Reticulitermes* (table 1).





Compound	Amount in the poison gland (μ g)	LD50 <i>Reticulitermes</i> (μ g/mg termite)	LD50 <i>Monomorium</i> (μ g/10 ants)
Pyr1. 	0.7	0.23 \pm 0.02	75.3 \pm 11.9
Pyr2. 	2.39	2.54 \pm 0.05	82.9 \pm 4.7
Pyr3. 	0.24	3.34 \pm 0.48	296.6 \pm 86.4
Pyr4. 	0.07	1.07 \pm 0.08	48.2 \pm 2.7

Table 1: Toxicity of pyrrolidines and pyrrolines

The more active compound is Pyr1 (2-(1hex-5enyl)-5(1non-8enyl)pyrrolidine).

The LD50 against Reticulitermes (0.2 μ g/ml) is lower than those of commercial insecticides (Nicotin 0.5, Methomyl 0.25). Moreover, this insecticidal activity is non-specific: a similar activity was obtained against other insects (Diptera, Lepidoptera, Orthoptera). Nevertheless, these compounds show no activity against the ants, specially considering the biological amounts in the venom bag. The emitters might be self-protected, or might detoxify quickly the venom. Those alkaloids can therefore be considered as powerful insecticides used as a chemical weapon by the ants.

As the venom is only laid down on the prey's cuticle, the toxins have to be able to cross the cuticle and specially the wax layer, in order to reach their target. This implies some adapted chemical properties, related with structures. Moreover, the contact with the prey is reduced to the deposit of the venom droplet on any part of the body. This enables the ants to avoid any dangerous contact or physical struggling.

This chemical strategy of aggression may represent a real advantage and a noticeable increase in the efficacy of predation.

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The Defensive Chemistry of Ants of the Genera *Tetraponera* and *Crematogaster*

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Stinging is probably the most noticeable defensive method utilized by the Formicidae. However, in many species, alternative defense mechanisms have evolved. We report here on the defensive chemistry of two species of ants, a Neo Guinean *Tetraponera* sp. (Pseudomyrmecinae) and *Crematogaster scutellaris* (Myrmicinae), collected in France. Both species do not sting but smear on enemies a contact poison with strong insecticidal properties (see Pasteels *et al.*, this volume).

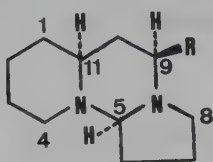
Tetraponera sp.

The venom of 250 workers was collected on bits of filter paper and stored in hexane. Rapid filtration of the hexane solution on alumina afforded 16 mg of purified extract. GC/MS analysis of this extract (25 m OV-1 column, 170°) indicates that it corresponds to a mixture of 8 closely related derivatives (T1 1%, T2 5%, T3 1%, T4 16%, T5 11%, T6 31%, T7 7%, T8 27%). Furthermore, as can be seen from table 1, the 8 derivatives are distributed into 4 pairs of isomers characterized by a nearly identical mass spectrum within each pair.

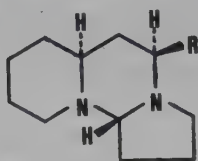
Table 1

Compound	M ⁺	Empirical formula
T1, T2	208	C ₁₃ H ₂₄ N ₂
T3, T4	222	C ₁₄ H ₂₆ N ₂
T5, T6	236	C ₁₅ H ₂₈ N ₂
T7, T8	250	C ₁₆ H ₃₀ N ₂

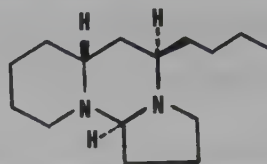
The same mixture was obtained by extraction of 1,250 whole ants after acid/base partition. Preparative GLC (SE-30 column, 150°) yielded 7 mg of T8, mp 40°; $[\alpha]_{579}^{+102}$ (c=0.15, CHCl₃). A careful spectroscopic study of T8, in particular by 2D ¹H/¹H and ¹H/¹³C NMR, led to the structural hypothesis 1, that was fully confirmed by X-ray diffraction analysis. By a combination of chromatographic methods, a few mg of compounds T3 to T7 were obtained in a pure state. Careful comparison of the spectral properties of T8 and of reference derivatives with those of T3, T4, T5 and T7 shows that T4 is the propyl analog of T8 (2), whereas T3, T5 and T7 must be represented by structures 3, 4 and 5, respectively, where the configuration of C-5 is epimeric with that of T4 and T8. Structure 6, proposed for T6, arises from an inversion of configuration at C-11 of the T4/T8 skeleton. Analyses of extracts from dissected poison and Dufour's glands have shown that these alkaloids originate from the poison gland.



T8 (1) $R = C_5H_{11}$
 T4 (2) $R = C_3H_7$



T3 (3) $R = C_3H_7$
 T5 (4) $R = C_4H_9$
 T7 (5) $R = C_5H_{11}$

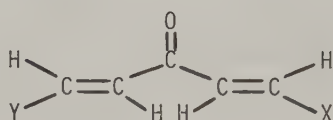


T6 (6)

Crematogaster scutellaris

Ants of the genus Crematogaster are unique among the myrmicines in that they use their Dufour's gland secretion as a defensive contact poison. Indeed, their spatulate sting is not a suitable injection device, but it would function ideally as an organ for topically applying venom. TLC analyses of the secretion indicate the presence of 5 major spots, corresponding to at least 5 components numbered CS1 to CS5 in order of increasing polarity. These components were separated on a Sephadex LH-20 column (eluent: $CHCl_3:CH_3OH:heptane$, 10:1:10). HPLC analysis of the major fraction, CS2, demonstrated that it is still a mixture of at least 6 compounds, differing by the position or the number of double bonds.

By a combination of spectroscopic and chemical methods, the structure of the 3 major derivatives of CS2, C(14%), D(44%) and F(27%) have been determined as 7a, 7b and 7c, respectively. The 2 other major fractions of the secretion, CS1 and CS5, are also mixtures of double bond isomers. They are structurally related to CS2, the sole difference being the replacement of the primary acetate group of the latter by an aldehyde (8a, 8b and 8c) or a carboxylic acid function (9a, 9b and 9c), respectively. To our knowledge, these derivatives are new natural products and constitute the first example of electrophilic poisons in the defensive secretion of an ant.



	X	Y		X	Y
<u>7a</u>	CH_2OAc	$C_{17}H_{33}, \Delta^{16}(Z)$	<u>8c</u>	CHO	$C_{17}H_{31}, \Delta^{14,16}(Z,Z)$
<u>7b</u>	CH_2OAc	$C_{17}H_{33}, \Delta^{14}(Z)$	<u>9a</u>	COOH	$C_{17}H_{33}, \Delta^{16}(Z)$
<u>7c</u>	CH_2OAc	$C_{17}H_{31}, \Delta^{14,16}(Z,Z)$	<u>9b</u>	COOH	$C_{17}H_{33}, \Delta^{14}(Z)$
<u>8a</u>	CHO	$C_{17}H_{33}, \Delta^{16}(Z)$	<u>9c</u>	COOH	$C_{17}H_{31}, \Delta^{14,16}(Z,Z)$
<u>8b</u>	CHO	$C_{17}H_{33}, \Delta^{14}(Z)$			

Chemical Defense by Contact poison in *Tetraponera* (*Pseudomyrmecinae*) and *Crematogaster scutellaris* (*Myrmicinae*)

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Chemical data on the poisons are given elsewhere (Daloze et al., this volume), additional biological informations are reported here.

TETRAPONERA.

This Neo-Guinean *Tetraponera* sp. (group *punctulata*) is unable to sting. While fighting with other ants, *Tetraponera* smears a secretion produced by the venom gland upon its enemy's integument. Even much larger and aggressive ants were visibly handicapped by the poison. They lost their hold and ran away with jerky and uncoordinated movements suggesting nerve poisoning. The major constituent of the poison, the alkaloid Tetraponerine-8, causes immediate paralysis when dissolved in methanol and applied topically on the cuticle of *Myrmica rubra* workers. Paralysis may last several hours, but ants never recovered after 24 h. LD_{50} was estimated to be 5.10^{-4} mg ($2.0 \cdot 10^{-9}$ M)/ant mg, which is low compared to a natural alkaloidal insecticide like nicotine, 4.10^{-3} mg ($2.5 \cdot 10^{-8}$ M)/ant mg. *Tetraponera*'s sting retains some of the primitive features characteristic of *Pseudomyrmecinae*. It is a powerful structure possessing barbs at the tip of the lancets and stylet. It is no longer a penetrating organ but rather a well adapted tool to deposit liquid on a surface. The lancets go beyond the stylet and diverge from each other like a drawing pen.

CREMATOGASTER SCUTELLARIS

C. scutellaris also smears its venom on enemies. Foe ants entering in contact with the secretion exhibit signs of distress. The poison is a complex mixture of C_{23} long chain derivatives characterized by the presence of a cross-conjugated dienone linked to either a primary acetate, an aldehyde or a carboxylic acid function. The poison originates in the Dufour's gland but only the stable acetates are secreted and stored in it. The more labile and electrophilic compounds are formed during the emission by enzymatic reactions. The venom gland contains the necessary enzymes. The secretions collected on bits of filter paper and immediately dipped in CH_2Cl_2 contain nearly pure acetates. The aldehydes and acids started to appear when the filter papers were kept in the open air. The alcohols are highly ephemeral. Aldehydes and acids appeared when Dufour's glands and poison glands were mixed together. Such reaction did not occur when Dufour's glands were crushed with pieces of fat body. LD_{50} for *M. rubra* after topical application was estimated as 1.10^{-2} mg/ant mg of the crude secretion (dry weight). The action of the venom was slower than with *Tetraponera*'s venom. The sting is much reduced and spatulate. The spatula is made by the stylet, flattened in the sagittal plane and somewhat twisted. The lancets are shother, flattened, and more or less confined between the stylet's fold.

The Major Component of the Poison Gland of the Leaf-Cutting Ant *Atta laevigata*

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The leaf-cutting ant *Atta laevigata* is a very important agricultural pest in Brazil. In the State of Minas Gerais it causes extensive damage to crops (OLIVEIRA, 1975). Behavioral studies in the field have centred around the trail pheromone. For many other species of *Atta*, compounds such as pyrrol and pyrazines have been described as components of the trail pheromone (TUMLINSON *et al.*, 1971; RILEY *et al.*, 1974). For the investigation of the poison gland compounds in *A. laevigata*, capillary gas chromatography and mass spectrometry was used with the system GC/MS HP-5996A. The column was a SE-30, 30m x 0.22 mm id fused silica type WCOT coupled to the mass spectrometer. A preliminary investigation of the crude extract of *A. laevigata* shows the presence of interesting substances such as, 1,7,7-trimethylbicyclo-[2.2.1]-hept-2-ene, 1-octanol, phenylacetic acid and methyl-4-methylpyrrole-2-carboxylate. After this first procedure, poison glands were dissected from the abdomen and extracted with CH₂Cl₂. The extract was injected in the GC/MS system and showed only the presence of the methyl-4-methylpyrrole-2-carboxylate.

The total ion current (TIC) of the crude extract of *A. laevigata*, showed large amounts of hydrocarbons and other compounds that, until now have not been able to identify.

In the TIC of the extract of the poison gland, there are only three peaks present. The peaks 1 and 2 are contaminants and peak 3 is the methyl-4-methylpyrrole-2-carboxylate as shown from the mass spectra.

In the present study the 3-ethyl-2,5-dimethyl pyrazine described as the trail pheromone for other species of ants (CROSS *et al.*, 1979; ATTYGALLE, A. MORGAN, E.D., 1984) was not detected. The biological function of the methyl-4-methylpyrrole-2-carboxylate has been investigated, and preliminary bioassays indicated that this compound is the main component of the trail pheromone of this species.

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The Contents of the Dufour Gland of the Ant *Pogonomyrmex occidentalis*

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The ant genus *Pogonomyrmex*, or harvester ants of North America, form large colonies which collect seeds from a defined territory around the nest (Cole 1968). This territory may cover many square metres and has permanent, branching trails which are patrolled by the worker, who defend it against incursion from other colonies or species. The Dufour glands and their secretion in *Pogonomyrmex* have not yet received much attention. Regnier et al., (1973) examined the secretion of *P. barbatus* and *P. rugosus*. They found homologous families of straight chain alkanes from C₁₂ and C₁₅ and three types of methyl-branched alkanes, and two dimethylalkanes. The major substance in *P. barbatus* was n-dodecane with 6-methylalkanes next. In *P. rugosus* the major peak obtained in gas chromatography was an apparently unresolved mixture of 6-methylundecane and 5-methylundecane. The species-specific nature of the contents of the Dufour gland of *P. occidentalis* (Cresson) and the existence of a new sesquiterpenoid compound discovered in the secretion are described here.

MATERIALS AND METHODS

A colony of *P. occidentalis* was collected at Denver, Colorado in August 1982 and transported live to Belgium, where the Dufour glands were removed, sealed in glass capillaries and sent to England where the gas chromatography was carried out. A further sample was collected at Denver in 1985, sent by mail to Athens, Georgia, where glands were dissected and sent to England for GC and to Germany for mass spectrometry.

Gas chromatography and mass spectrometry were carried out as described in other publications from the group (C.F. Ollett et al., 1986). Essentially, single Dufour glands from workers were sealed in glass capillaries and heated in the gas chromatograph, the capillary was crushed and the volatiles chromatographed without the use of solvents (Morgan and Wadhams, 1972a). For mass spectrometry, the same technique was used, with the mass spectrometer directly coupled to the gas chromatograph.

RESULTS

The principal substances in the Dufour gland reservoir were n-alkanes from dodecane to nonadecane, methyl-branched alkanes from C₁₃ to C₁₅ and 3,5-dimethyltridecane. The total mean amount in the glands of the first sample collected was 527 ng and 70 ng in the second. This kind of variation in amount is not uncommon, since the amount of material in any one worker's gland is very variable, though the composition remains relatively constant. n-Pentadecane was the principal constituent (29% in the first, 32% in the second) with n-tridecane second (14.6% in the first, 11.6% in the second). Next came heptadecane, tetradecane and dodecane. The branched alkanes were all minor constitu-

ents, with 6-methyldodecane as the most abundant one (3.7%). There were two alkenes, octadecene (7%) and nonadecene (7%), but most interesting was the presence of a bishomofarnesene compound, $C_{17}H_{28}$, representing 8% of the total. This is a new compound, having a different mass spectrum from the bishomofarnesene of Myrmica Dufour glands (Morgan and Wadhams, 1972b); Attygalle and Morgan (1982; 1984), and from the bishomofarnesene of Monomorium pharaonis and Solenopsis geminata Dufour glands (Attygalle and Morgan, 1987).

Though the mixture of Dufour gland hydrocarbons is probably species-specific and is certainly different from those of P. barbatus and P. rugosus (Regnier et al., 1973), the presence of the relatively unstable sesquiterpenoid is probably more interesting as a possible pheromone and requires behavioural testing. From the figures in Regnier et al. (1973), the same substances may well be present in P. barbatus and P. rugosus but were not identified.

This substance, highly unstable in air when pure, will be stabilized by solution in the hydrocarbons and will have a limited lifetime for conveying a chemical message. Its stability under the conditions of sampling attests to the reliability of this method of collection and analysis by sealing in capillaries and direct injection into a gas chromatograph (Morgan and Wadhams 1972a).

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The Mandibular Gland Secretion of the Ant *Manica rubida*

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The mandibular gland secretions of ants are generally known to play an important role in their "alarm-defence" behaviour (Maschwitz, 1964). The mandibular gland chemistry of two North American species of *Manica* have been studied (Fales et al., 1972). The opportunity to study *Manica rubida* Latr., the only Eurasian representative of the genus *Manica*, aroused our interest, as an extension of our studies on chemistry and ethology of exocrine secretions of ants.

MATERIALS AND METHODS

A nest of *M. rubida* was collected at St André-en-Vivarais (Ardèche, France), and maintained in the laboratory in artificial nests partly filled with moistened plaster. Worker ants were anaesthetised by exposure to carbon dioxide and the mandibular glands were removed by dissecting the heads under a binocular microscope. The excised glands or individual heads were sealed in solid-sampling vials in preparation for gas chromatography (Morgan and Wadhams, 1972). A 25m x 0.22mm fused silica column coated with SP-2340 was used for GC analysis: 2 min at 60°C, 60-195°C at 4°/min. Gas chromatography-mass spectrometry was performed on a Finnigan 9502 GC linked to a 3200E quadrupole mass spectrometer.

RESULTS

GC analysis of volatile compounds from an individual head of *M. rubida* worker revealed the presence of several components (Fig. 1). The major component, peak 11 of Fig. 1, showed the same mass spectrum and retention time as those obtained from an authentic sample of (4E)-4,6-dimethyl-4-octene-3-one. This compound, manicone, has been identified in two North American species of *Manica* as the major volatile component in the mandibular gland secretion (Fales et al., 1972).

The mass spectra of peaks 10, 11, 12 and 13 showed similar characteristics. The parent ions were found at m/z 140, 154, 168 and 182, respectively. From the spectra, it was evident that these four compounds were members of a homologous series. This conclusion was supported by their gas chromatographic retention behaviour.

A homomanicone, (4E)-4,6-dimethyl-4-nonene-3-one, was synthesized. The retention time and mass spectrum of the synthetic compound was identical to those of ant homomanicone [m/z 168(40%, M⁺), 153(4), 139(100), 125(26), 111(10), 97(22), 69(98), 55(52), 41(78)]. By analogy, the next homologue of the series is expected to be (4E)-4,6-dimethyl-4-decene-3-one.

The structure of normanicone is not yet clear. Two isomers, (4E)-4,6-dimethyl-4-heptene-3-one and (4E)-4-methyl-4-octene-3-one, were synthesized. However, their spectra do not appear to correspond to that of ant normanicone.

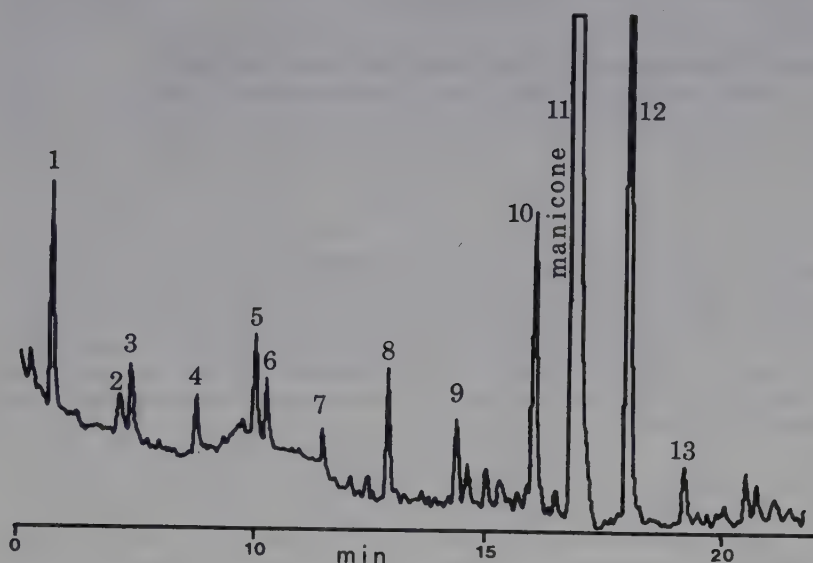


Fig. 1. -- Reconstructed gas chromatogram of the volatiles from the head of a *M. rubida* worker. 1 = acetaldehyde, 2 = 2-methylpropanal, 3 = acetone, 4 = 3-methylbutanal, 5 = unknown, 6 = dimethyldisulphide, 7 = 4-methyl-3-hexanone, 8 = 4-methyl-3-heptanone, 9 = 4-methyl-3-octanone, 10 = normanicone, 11 = manicone, 12 = homomanicone, 13 = bis-homomanicone.

The synthesis, biological activity and a detailed analysis of the mass spectra of these manicones will be published elsewhere.

The other compounds identified as volatile components in the mandibular gland secretion are shown in Fig. 1. Similar compounds are frequently encountered in ants (Attygalle and Morgan, 1984). In addition to the more volatile compounds, a series of higher-boiling compounds appeared later in the chromatogram. However, these compounds, mainly hydrocarbons, did not appear in the chromatographic profiles obtained from excised glands.

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Chemical News from Bees and Ants

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Acetogenins in cephalic secretions of workers of Meliponinae

The mandibular gland secretions of stingless bees represent complex mixtures of simple hydrocarbons, methylketones, methylcarbinols and esters most of which originate from the acetate pool^{1,2}. Straight chain alkanes, primary alcohols, carboxylic acids and methylketones represent more or less ubiquitous compounds which often form the "backbone" of stereotypic mixtures showing some species specific variation.

Methylcarbinols and their esters may be used for the formation of unique enantiometric mixtures. In many cases, esters add decisively to the complexity of the signals and account for a species specific "fine tuning". In fact, esters form a particularly versatile class of compounds through variation of chainlength at both sides of the functional group as well as through insertion of double bonds; they frequently represent true qualitative differences in different species.

Compounds	Species					
	1	2	3	4	5	6
2-Heptanone	m	m	t	t	m	M
2-Nonanone		t	t			m
2-Undecanone		t			t	t
2-Tridecanone	m	m			t	m
2-Pentadecanone	m					t
2-Heptanol	M	M	M	M	M	m
2-Octanol	m	t		m	m	
2-Nonanol	t	t		m	t	t
2-Undecanol						t
2-Tridecanol		t			m	t
2-Heptyl acetate	m	m		t	t	
2-Heptyl butyrate		m	t			
2-Heptyl hexanoate	m	t	t	t	t	t
2-Heptyl heptanoate M				t		
2-Heptyl octanoate		t		t	t	
Hexyl hexanoate		m	m	t		
Octyl hexanoate			M		t	
Decyl hexanoate			m	M		m
Hexyl octanoate				M		
Octyl octanoate			M	M	M	t

M = main, m = medium, t = trace component

1 = *Melipona marginata* 2 = *Melipona quadrifasciata*

3 = *Tetragona clavipes* 4 = *Trigona hyalinata*

5 = *Trigona spinipes* 6 = *Scaptotrigona bipunctata*

Esters which carry the functional group towards the middle of the molecule follow the chemical principle of bees wax; higher volatile members obviously are used for odour communication while less volatile compounds may play a role as tactile stimuli and high molecular compounds form nest building material³.

In the table, selected compounds from the cephalic secretions of 6 Brazilian stingless bee species are compiled. The results were obtained with old workers, and it should be noted that the composition may vary with age⁴. Though basically very similar, the bouquets are clearly species specific; actually the compositions are much more complex than is shown here. 2-Heptanol is dominating in most species; *Scaptotrigona postica* contains (S)-(+)-2-heptanol in 95% optical purity. 2-Heptyl esters seem to be particularly important in odour communication of Meliponinae⁵.

Volatile compounds from *Formica* spp.

Pentane extracts of heads of workers from several *Formica* species contain methyl 3-ethyl-4-methylpentanoate and 3-ethyl-4-methylpentanol. The compounds originate from the mandibular glands and reach relatively high concentrations

in queens⁶. They may represent a "Formica-badger" with a certain "queen-significance".

The abdominal fraction (Dufour's gland secretion) of workers is largely dominated by straight chain, odd numbered hydrocarbons. Among the low boiling compounds undecane forms the main component^{7,8}, while the concentrations of its bishomologues, C-13 to C-17 decrease. The amounts of higher boiling hydrocarbons may increase again starting from C-23; alkenes prevailing.

Unbranched esters form another class of Dufour's glands constituents, hexadecylformate being a significant component in many species. Though these multicomponent mixtures often show little qualitative variation, frequently species-specific quantitative differences can be detected which are useful in chemotaxonomical investigations. However, it may be useful not only to compare workers but also males and females in different physiological stages.

Extracts from the abdomina of workers of *F. aquilonia* from South Finland and from those of a closely related sympatric species, preliminary termed "*F. polyctena*" could not be distinguished by glc. In contrast, extracts from queens showed clear differences in the relative proportions of the formates and acetates of Z-5-tetradecenol and Z-5- and Z-7-hexadecenol. The hydrocarbon patterns proved to be identical in all respects. It appears that specific differences in the patterns of oxygen-containing compounds (esters) are more pronounced than in the hydrocarbon fractions.

Comparison of extracts of workers of *F. uralensis* from South Finland and a small population from South Germany (a relict from the glacial time) revealed striking similarities: no qualitative differences could be detected, and the hydrocarbon patterns proved to be very similar. The species shows an interesting group of esters: formates, acetates and trace amounts of propanoates of decanol, undecanol and dodecanol. While in the Finnish population relative proportions between the formates and the respective acetates reach about 1:5-decyl acetate forming the main component within the group - the German population contains only trace amounts of formates. Relative proportions of decyl acetate, undecyl acetate and dodecyl acetate were similar in both populations. The acetates are present in *F. sanguinea* and *F. rufibarbis*, too⁷. The data discussed above represent results of several preliminary analyses which need further statistical support. They show, however, that chemical analysis may be a useful tool in the taxonomy of *Formica* spp.

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Chemical Communication in the Primitive Cockroach *Cryptocercus punctulatus*

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Cryptocercus punctulatus, a wingless wood eating cockroach, is considered the most primitive existant number of the Blattaria and is believed to be related to the ancestral stock that gave rise to the termites. It remains a relatively unknown organism and its behavior and intraspecific relationships are still unclear (Nalepa 1984).

Males and females possess tergal glands which display an important dimorphism in their position, their morphology, and in the chemical composition of their secretion. In many other species of cockroaches, the tergal glands produce chemical signals during the precopulatory behavior; though little is known of the sexual behavior of this cockroach, such a role must be considered.

The females tergal glands of C.punctulatus, are characterized by an unusual association of several types of glandular cells and by the secretion of specific alcohol.

One hundred of females of this rather rare insect were collected in two years and only 40 ug of this alcohol were purified by preparative G.C.. The data obtained through G.C.-M.S., G.C.-F.T.I.R. and N.M.R. allow us to propose the following structure : 6-(1-hydroxy ethyl) 4,5,7-trimethyl 2,4-nonadiene; $C_{14}H_{26}O$; M.W. = 210.

The identification of this new compound supports the hypothesis that the specificity of the chemical signals used by the cockroaches during the sexual behavior is rather due to specific complex compounds than to a specific mixture of non specific compounds (Brossut, Sreng 1986).

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Lability of the Mandibular Gland Signal of Three Races of African Honey Bees

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The mandibular gland signals of both workers and queens of the honey bee races Apis mellifera capensis, scutellata and mellifera have been shown to change with age and social milieu (Crewe and Velt-huis 1980, Crewe 1982). Queenless capensis workers rapidly develop into 'false' queens with queenlike mandibular gland (MG) contents. Crewe (1984) has suggested that the signals of capensis queens and workers are different from those of other races as a consequence of thelytokous parthenogenesis in the workers. However, an alternative hypothesis is that the expression of a specific MG secretion is related to the speed with which ovarian development occurs in queenless workers. To test this hypothesis we investigated the MG signals of A. m. intermissa, a race of African honeybees in which worker ovarian developments occurs rapidly (Ruttner and Hesse 1981).

MATERIALS AND METHODS

Queens and workers used in these studies were A. m. capensis, scutellata, mellifera and intermissa. Laying workers of capensis were produced by placing 1 day old individuals in cages with 30 scutellata workers. Similarly, 2 day old intermissa were placed in cages with 80 carnica workers. Individuals from these cages were harvested at regular intervals for chemical analysis and assessment of ovarian condition. Queens and virgin queens were maintained in nucleus colonies and harvested as above.

MG gland contents was analysed by removing heads, and placing them in dichloromethane. The extracted contents of the glands was treated to produce TMS-derivatives of the fatty acids. These were then analysed using fused silica capillary gas chromatography.

RESULTS AND DISCUSSION

The mandibular gland contents of workers of scutellata are dominated by the production of the acid (E) 10-hydroxy-2-decenoic acid (10HDA), and laying workers occasionally produced (E) 9-keto-2-decenoic acid (9ODA) as a minor component of the mixture. Analysis of capensis workers confirmed that after 2 days 9ODA starts to predominate in the mixture and 10 day old workers produce a mixture of MG chemicals characteristic of queens. Table 1 presents the data on the analysis of the composition of the MG gland contents and shows that in both intermissa and scutellata the contents undergo a change from a relatively worker-like composition to a situation in which 9ODA predominates. A similar process of development is not evident in capensis. There is thus a clear distinction between the races, with intermissa showing similarities in composition to mellifera MG contents.

The MG contents of 97 workers of a variety of ages and ovarian developmental states were analysed, none were found to have anything equivalent to the mixtures produced by capensis workers. The results are presented in Table 2.

Table 1. - Percentage composition of the major components of mandibular gland secretions of A. m. intermissa, scutellata and capensis laying and virgin queens.

Queen	n	8OHOA	9ODA	9HDA	HDA	10HDA
<u>intermissa</u>						
Virgin	9	6.70	25.62	50.95	1.60	15.12
Laying	8	4.14	49.76	23.31	8.69	14.11
<u>scutellata</u>						
Virgin	5	4.48	38.89	13.10	6.74	31.94
Laying	15	4.77	65.39	14.42	3.64	8.05
<u>capensis</u>						
Virgin	27	2.58	80.09	11.05	0.00	3.83
Laying	6	3.00	84.83	9.78	0.00	1.01

8OHOA= 8 hydroxy-octanoic acid; 10HDA= 10 hydroxy-decanoic acid and 9HDA=(E) 9 hydroxy-2-decenoic acid.

Table 2. - Amounts (ug) of the major components of mandibular gland secretions of A. m. intermissa queenless workers of various ages in days.

AGE(n)	8OHOA	9ODA	9HDA	10HDA	10HDA
8(4)	7.96	0.00	7.99	1.29	01.65
12(3)	3.88	0.09	6.41	3.19	00.72
50(3)	0.95	0.33	2.46	6.41	13.75

Although intermissa workers can develop laying workers quickly, there is no switch to the production of queenlike MG secretions such as is found in capensis, and their queens produce secretions that are similar to those of races in which thelytokous parthenogenesis is absent.

The situation in capensis is unusual: the workers produce queen-like signals and virgin queens do not show a change in signal composition with age. The unique features of these signals may be functionally related to the worker thelytokous parthenogenesis. If true, then the simple 1 locus with 2 alleles model for parthenogenetic reproduction in worker honey bees (Moritz 1986) may be too simplistic. This question is being studied in hybrids of capensis and scutellata.

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A Honey Bee Queen Pheromone that Repels Workers

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Natural queen production in honey bee colonies results in the production of several virgin queens, only one of which ultimately becomes queen of the parental nest. As a consequence of extreme polyandry and patterns of sperm use by queens, colonies are composed of a large number of individual subfamilies (patriline). The workers of each subfamily share a mother queen and father drone. Kin and natural selection theory predict that if workers have the capability to distinguish among individual workers and virgin queens belonging to different colony subfamilies and if the reduction in colony fitness is not too great as a consequence of subfamilial competition, then worker-worker & worker-queen conflict should occur over which virgin queen survives and reproduces in the parental nest.

Queen-queen conflict for colony dominance is well known for honey bees. Worker-worker and worker-queen conflict have recently been studied under laboratory conditions that have demonstrated that workers have the perceptive abilities necessary for conflict to evolve. However, further studies under less contrived conditions are still necessary to determine the adaptive significance, if any, of this behavior.

We present the results of laboratory experiments that demonstrate that young virgin queens produce a pheromone that is released with rectal content (Post et al. 1986). The behavioral response of workers to the feces of queens suggests that this material may be involved in resolving episodes of worker-queen and queen-queen conflict (Page and Erickson 1986).

MATERIALS AND METHODS

Virgin queens were produced by standard queen-rearing methods. Sealed queen cells were placed into clean, glass vials and maintained in an incubator at 34°C until the queens emerged. Four virgin queen treatment groups were established: Virgin queens remained in the glass vials in the incubator for 2-3 days and were provided with (1) sugar candy and the residual royal jelly in the queen cell, or (2) were provided with sugar candy only. Other virgin queens were placed into individual screen-wire cages and then placed into a queen-holding colony where they were fed by workers for (3) 3-4 days or (4) 2-3 weeks. An additional 2 worker treatments were established by placing newly emerged worker adults into clean, glass vials corresponding to virgin-queen treatments 1 and 2.

Groups of 10 super-sister workers were collected from colonies into 235 ml cardboard cups (observation arenas). A 6.0 cm diameter piece of filter paper with a 1.8 cm diameter circle marked in the center was taped to the bottom of each arena. Arenas were taken into the laboratory for behavioral assays. Prior to each assay, the feces

of a treatment queen was collected. For each replicate, a test and a control arena were paired. The behavior of workers within an arena was observed for 60 secs then for an additional 60 secs following the placement into the center of the circle of 10-30 ul of rectal content from a single queen (treatment) or an equivalent quantity of distilled water (control). Treatments and controls were alternated. The number of workers walking through the circle during each 60 sec period, the number of workers on the bottom of the arena after 60 secs, and the number of workers autogrooming after 60 secs were quantified for each of the 92 replicate sets of treatments and controls.

RESULTS

Rectal content of virgin queens that are 2-4 days old repels workers and releases autogrooming behavior. There was a significant decrease relative to the control groups in the number of workers that walked through the circle during the 60 sec observation period and a decrease in the number of workers that autogroomed after 60 secs following introduction of feces of queens from treatments 1, 2, and 3 ($P < 0.01$, chi-square contingency table analysis, for each). Fewer workers remained on the bottom of the arena, relative to the controls, for all treatment groups, however, the difference was statistically significant only for treatment 1 ($P < 0.01$). Feces of queens 2-3 weeks old (treatment 4) did not elicit any demonstrable response from workers. Feces collected from workers that were treated identically to queens of treatments 1 and 2 did not elicit a demonstrable response (63 replicate sets of controls and treatments).

In cage studies Page and Erickson (1986) reported that virgin queens release large quantities of feces during agonistic interactions with workers and during dominance fights with other queens. Frequently, workers stopped their agonistic behavior and, instead, engaged in autogrooming. When queens fight, the rectal content of one queen is frequently excreted on the body of the other. Workers are then repelled by the contaminated queen. Workers appear to be highly attracted to very small quantities of feces that are released by virgin queens. The chemical composition of virgin queen feces is being determined. The adaptive significance (if any) of feces pheromone of queens remains to be demonstrated.

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Functional Evolution of Dufour's Gland Secretion in Bees

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Dufour's gland of bees is often well developed and rather diverse in its biosynthetic capabilities. In ground dwelling bees the glandular secretion is utilized for creating a hydrophobic brood cell lining in order to provide the developing larva with a constant environment and to protect its food from microbial and fungal attacks (Hefetz et al., 1979). In addition, in some species the secretion may be also used for marking of the nest entrance. Problems in the exact location of the nest often encountered by ground dwelling bees become even more acute in species, like *Eucera palestinae*, that nest in dense aggregations. Foraging bees usually enter the right nest entrance but sometimes mistakes occur, in which case the bee realizes at the nest entrance that it is the wrong nest, comes out and walks around until she finds her own nest. Since in this species Dufour's gland secretion is used for lining cells (Shimron et al., 1984), it seemed logical that the bees use the same secretion as a nest marker. One of the features of a nest marker is that it has to convey individuality. Thus, exposure of the bee to a strange conspecific secretion, in contrast to its own secretion, should elicit a searching behaviour. In order to demonstrate that such individuality of the nest scent exists, bees were brought to the laboratory and their Dufour's was carefully removed and extracted. The Dufouriectomized bees were then returned to their nest and only the bees that demonstrated normal foraging behaviour the next day were tested. The tests included exposure of the returning bee to either a single strange Dufour's gland or to her own secretion. While the bees were confused when a strange Dufour's scent existed at the nest entrance, they behaved perfectly normal when exposed to their own Dufour's scent (Shimron et al., 1984). Thus, each bee has an individual Dufour's odour and can distinguish self from non self.

The same phenomenon of marking the nest entrance is apparent in the halictine *Evyllaesus malachurum*. In this species, as in *E. palestinae* the bees use Dufour's gland for lining the brood cell as well as for marking their nest entrance. Behavioural observations point to the fact that at least at close range, the scent at the nest entrance is important in locating the nest. This is true both at the onset of the season when the queen forages for food and later on when the workers take up foraging duties. If the scent at the nest entrance conveys individuality, then this should be also reflected in the chemical composition of the glandular secretion. Expression of individuality via a pheromone within a species is usually achieved by changing the relative intensities of the various pheromonal components. To demonstrate that for *E. malachurum*, individual glands were compared using cluster analysis of cases by which each of the secretions is compared to all the others based on the relative amounts of the components present in the secretion. Using this procedure, nestmate (considered as sisters) and randomly collected bees were compared (Hefetz et al., 1986). While each bee has a distinctive composition it is evident that nestmates are more

similar with this respect to each other than randomly collected bee. Preliminary behavioural experiments indeed suggest that Dufour's secretion is at least in part responsible for supplying the odours involved in nestmate recognition. It is possible, therefore, that this degree of similarity in the odours is indistinguishable by the bees and that discrimination is based on self vs. non self scents. It also follows that kinship between nestmates facilitates the discriminatory process.

A possible evolutionary scenario for Dufour's gland secretion in bees can be discerned: Changing the feeding behaviour of the larvae necessitated a hydrophobic cell lining and Dufour's being adjacent to the pygidium became hypertrophic and produced the necessary chemicals. Problems in nest location may have selected in favour of nest entrance marking, and Dufour's gland secretion was suitable for that function as it was already used to line the cells and may have assisted in the location of the opened cell in the nest. Furthermore, nest markers have to be close range semiochemicals in order not to lure kleptoparasites from a distance. Dufour's gland secretion fulfils exactly this expectations since chemicals with low volatility are required in order to create a hydrophobic lining. Thus, the evolutionary step required was to develop the receptors for these compounds in order to adapt the secretion for nest marking. Having the self communicative function established, it would take only a small evolutionary step to adapt it for intraspecific communication, probably nestmate recognition.

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What Information Do Social Halictine Bees Encode In the Amount of the Macrocyclic Lactone Pheromones?

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Males and females of the primitively eusocial bee Lasioglossum (Dialictus) zephyrum discriminate among genealogically related and unrelated females during mating behavior and social interactions within the nest. Information on kinship is encoded in a pheromone mixture produced by the females (Smith, 1983). In both L. zephyrum and L. (Evylaeus) malachurum, related females are more similar to one another than are unrelated ones with respect to the mixture of macrocyclic lactones found in the Dufours gland (Hefetz, this volume). However, at least with L. zephyrum, one highly variable factor among females is the amount of pheromone released, which varies randomly with respect to genealogical relationship. Production of the pheromone is probably energetically costly. Here we report on a possible communicative role for release rate variation among female L. malachurum, which includes communication of size in intraspecific fighting contests.

Materials and Methods

Spring queens (solitary stage of the life cycle) of L. malachurum were collected in May of 1984 and 1985 around Tübingen, West Germany. In 1984, the bees were first killed by freezing, and then extracted whole in 300 μ l of spectral grade hexane for 48 hrs. They were then removed from the solvent and fifteen morphological characters were measured. In 1985, queens were first used in a behavioral assay in which two individuals were first placed into a plastic tube, which was then bent around so that the two ends could be connected. The two individuals were thereby forced to interact several times, and their behaviors were recorded. After observation, both bees were placed into silanized glass vials for up to six hours for collection of their pheromones. All vials were then washed with 300 μ l of hexane. In 1986, the behavioral observations were repeated, however, this time the smaller of the two bees was treated with either acetone or acetone containing 2 μ g of a synthetic lactone mixture made to match that of an average sample from 100 bees. All extracts were analysed on an HP5880 GC-integrator system fitted with an SE-54 capillary column. Ethyl stearate was used as an internal standard for quantification.

Results and Conclusions

No significant morphological allometry was found either within queens or between queens and workers of L. malachurum. Queens are on average 1.1 to 1.3 times larger than workers, although there is some overlap - the smallest queens are as large as the largest workers. However, queens produced four times as much material in the Dufours gland as workers. Furthermore, the amount of the Dufours secretion a queen contains is directly correlated with her size. No such relationship exists, however, for workers; that is, larger workers contain as much of the secretion as smaller ones.

When queens are placed together in the tubular arenas fighting always occurs. One measure, termed C-posture, where the bee bends the abdomen around such that the sting and mandible are presented simultaneously, was observed in most bouts, and was more frequent in the early stages of fighting. The larger of the two bees escalates the contest (by C-posturing) more often than the smaller. The conditional probability of C-posturing (given that an interaction occurs) for the larger was 0.11 and for the smaller 0.03 ($p < 0.05$). When the smaller of the two bees was treated with acetone only, the pattern remained the same - 0.16 and 0.06, for large and small bees respectively. However, when the smaller bee was treated with the acetone/lactone mixture, both bees C-postured equally frequent - 0.06 for both.

No relationship existed between size and release rate. Queens released on average the same amount as workers (0.40 and 0.34 ng/min, respectively).

Queens of L. malachurum can therefore use size as a cue for settling intraspecific fighting. Furthermore, the lactone pheromone can serve as a cue. However, because the release rate is not correlated with size, the pheromone may be used in conjunction with other cues; for example, acoustic or tactile cues during fighting.

The degree to which queens and workers differ with regard to size varies considerably within the Halictidae. Furthermore, the species within this group face largely the same parasites and predators while there is considerable variation in the lactone defensive secretions. We therefore propose that size differences between queens and workers and variation in defensive secretion arose through intraspecific fighting contests among queens in the early flight season, a phenomenon that we observed repeatedly with L. malachurum.

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Morphology of Pheromone Glands in *Meliponinae* (Hymenoptera, Apidae)

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Exocrine glands of ectodermal origin, potentially or certainly producers of pheromones, are quite frequent in bees. They may be located in the head, thorax or abdomen (Cruz-Landim, 1967; Cruz-Landim et al., 1980a and b; Mota, 1982).

MATERIAL AND METHODS

The exocrine glands of males, workers and queens of several meliponine species were studied. The glands were dissected (their localization and anatomy determined) and fixed as usual for light and electron microscopy.

RESULTS

Figure 1 represents an idealized bee, where all glands found to date in different castes and sexes of meliponines are represented. Only the mandibular glands are certainly producers of pheromone (Blum, 1974), but the tergal and Dufour glands can also be considered so, by analogy with other hymenopterans.

The glands of meliponines are constructed in two different patterns: 1) glandular cells bearing special structures for secretory discharge; 2) glandular cells without these (Fig. 1). The first type is formed by glandular cells that possess an intracellular canal for collection of secretions (Fig. 1c). Depending on the number and arrangement of these cells, they can form an epithelium or remain as isolated secretory units. The mandibular glands as well as most of the tergal glands are formed by this type of cell. The canal within the secretory cell is formed by a cell membrane invagination and has a cuticular lining. This intracellular canal is connected with an extracellular one and through this to the secretory discharge point (tegument in the case of tergal glands or reservoir in the case of mandibular glands). The second type appears as a simple modification of the epidermis, as in the gland from tergite III, or as a simple epithelial structure, as in the Dufour gland.

The cellular components in these cells vary according to the gland and from them we can get an idea of the nature of the secretion produced. The mandibular gland cells in newly emerged meliponines present great cytoplasmic basophily due to their richness in polysomes, but in the secretory phase they are acidophilic, and only a few profiles of rough endoplasmic reticulum were seen. The Golgi apparatus is detected only rarely. The secretion appears in the form of vacuoles of low electron density and tends to be accumulated around the intercellular canal.

The tergal glands present almost the same characteristics, except that the smooth endoplasmic reticulum is more conspicuous in this case, and the secretion forms granules of medium electron density. In the glands resulting from epidermal transformation, smooth endoplasmic reti-

culum predominates, either in the wax glands of workers or in the epidermal glands of the queen's tergite III.

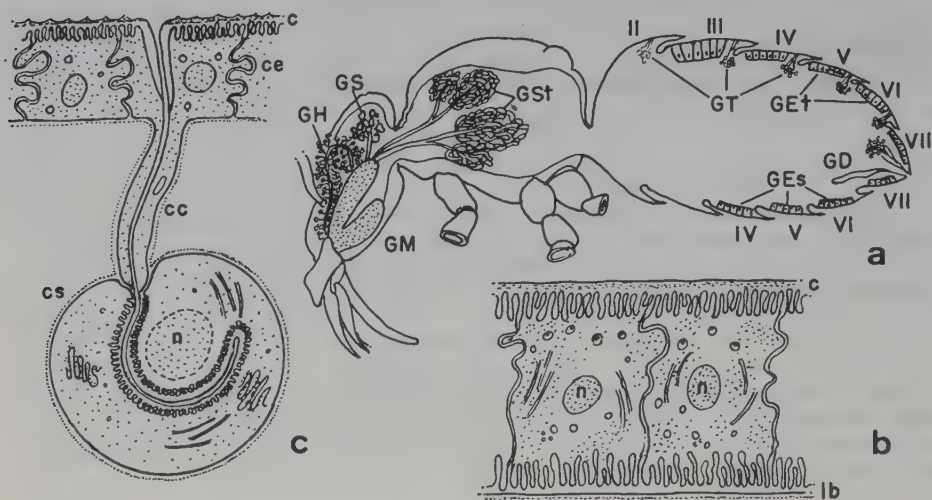


Fig. 1a. Localization of exocrine glands in meliponines. b. Epidermal gland from tergite III (queens) and tergites IV, V, VI and VII (workers wax glands). Secretory units found in mandibular or tergal glands from queens (tergite III to VIII and tergite II for some workers and males). GH. hypopharyngeal gland; GS. head salivary gland; GM. mandibular gland; GST. thoracic salivary gland; GT. tergal gland; GET. epidermal tergal gland; GD. Dufour gland; GES. epidermal sternal glands; c. cuticle; n. nuclei; cs. secretory cell; cc. canal cell; ce. epidermal cell; bl. basal lamina canal.

DISCUSSION AND CONCLUSIONS

The glandular cells of meliponines, candidates for pheromone production, present a structure that suggests a nonproteic nature of secretion as expected. The prevalence of smooth endoplasmic reticulum and a Golgi underdevelopment probably indicate secretion of lipid nature. The presence of intracellular canals for collection and release of secretions may indicate a product potentially dangerous to the glandular cell in the mature state, or cuticular permeability difficulties in its elimination. Finally, the variations in gland occurrence in males, queens and workers are caused by functional differences (between queens and workers), as well as genotypic ones (between males and females).

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Class Three Glands in *Vespa crabro*: an Ultrastructural Comparison between Venom and Sternal Glands

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Noirot & Quennedey (1974) provided convenient criteria for the analysis of subcellular specializations devolved to the collection and release of secretions from insect exocrine glands of ectodermal origin. Following their concepts we compared the ultrastructure of the venom (filamentous) gland and the exocrine organ in the 7th abdominal sternite (sternal gland), which produces a possible repellent, in workers of *Vespa crabro*. Similar functional units (organules) are found in venom and sternal glands, consisting of secretory cells and two types of accessory cells. These latter produce the duct cuticular wall (canal cells in both organs) and the cuticular layers where the secretion collects (squamous cells of the venom gland intima, and hypodermal cells in the sternite). Specializations for collection and release of the two secretions follow the same architecture. The secretory cells possess a deep "reservoir" to hold the end apparatus, consisting of branched, cuticular canalicules. Their wall (30-40 nm thick) is riddled with numerous pores, filled with a filamentous cuticle. The reservoir in the venom producing cells has an even deeper chamber lined with microvilli, which is a common feature in hymenopteran filamentous glands, but was not found in the hornet sternal glands we observed. However, the reservoir may vary greatly in appearance, according to its functional stage. In sternal gland Anlagen of *Polistes* we observed an ephemeral microvillous chamber, and in the mature glands a wide range of modifications in both width and shape of the reservoir following their secretory cycle. The synthesis machinery consists of rough reticulum and Golgi bodies, but signs of metabolic stasis may prevail in sternal glands. The venom producing cells adhere together by means of desmosome-like structures, possibly derived from symmetric coupling of basal hemidesmosomes, which can also be seen on the surface of sternal gland cells. Septate junctions hold the secretory cells to the cells of the duct, which arises from the end apparatus. Its non-fenestrated wall (100-130 nm thick) merges with the intima in venom filaments and the cuticle layer on the sternal hypodermis. Ultrastructural findings emphasize the role of cuticle-lined compartments in the collection and release of noxious substances through competent cells. Finally, since development of the elaborated pathways we have described requires proper interactions between specific cell components during organule morphogenesis, the identical patterns in the end apparatus of venom and sternal glands confirm their homology, as suggested by their common ectodermal origin.

Structure and Ultrastructure of the Dufour's Gland in *Parischnogaster* (Hymenoptera, Stenogastrinae)

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Stenogastrinae wasps produce large amounts of a milky, gelatinous substance which the females collect from the tips of their abdomens and use for several purposes in their reproductive and social biology. Studies on the anatomy of the female abdomen suggest that Dufour's (or the alkaline) gland is the site of production of this substance. Accordingly, we carried out a morphological study on the gland in *Parischnogaster alternata* and *P. mellyi* to verify this hypothesis and to correlate ultrastructural findings with a preliminary analysis of the composition of the product. The alkaline gland in *Parischnogaster* is a tapered tubule, consisting of a proximal stalk and a distal bulk, with a funnel-shaped neck inbetween. Both structure and ultrastructure agree with its gross morphology. The single-layered epithelium of the gland is columnar in the stalk, then becomes cuboidal in the bulk, thus contributing to the impressive enlargement of the lumen. Myocytes enwrap the gland and form an interrupted layer round the stalk and an almost continuous sheath distally, until they merge with the muscle complex at the tip of the abdomen. Throughout the gland, cell membranes are engaged in elaborate relationships, both mutually and with the hemolymph environ (via the basal lamina) and contribute to the septate junctions. Furthermore, thickenings on the peripheral plasma membranes are visible in the bulk where bundled microtubules converge, to form the cytoskeleton inside interwound cell processes. Organelles, possibly related to synthesis activity, consist of minute Golgi saccules and smooth reticulum strands, neither of which display any evident polarity. Patterns of secretory activity related to apocrine mechanisms prevail in the stalk. Fragments of cytoplasm are obvious inside the interstice between the epithelium and the cuticle lining of the lumen (intima) and contain myelin-like whorls of membranes. Discrete masses of amorphous material also occur, which flow into the lumen through pore channels in the intima. The secretion accumulates in the lumen and appears as a two-phase system, consisting of thin electron dense aggregates in an amorphous background. Present findings indicate that the alkaline gland in *Parischnogaster* may produce, store and discharge large masses of a secretion, which seems to correspond to the abdominal substance. The absence of arrays of rough reticulum excludes the synthesis and segregation of proteins in the secretory process. This agrees with biochemical tests on the substance but casts doubts on previous suggestions that it serves as a convenient food stuff for the larvae.

Gastral Exocrine Glands in Ants - Functional and Systematical Aspects

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In the last years a great variety and an unexpectedly high number of gastral intersegmental glands and glands associated with the sting apparatus were found (Hölldobler & Engel 1978, 1982, Kugler 1978, Jessen et al. 1979, Jessen & Maschwitz 1983, Jessen 1984).

Although a pheromonal or allomonal function for some of these glands has been documented by various authors (see Hölldobler 1982), the function of many others still remains unknown. In this paper a functional-morphological interpretation of these glands is given.

Phylogenetical conclusions according to the problem of a mono-, di-, or triphyletic origin of the army ants are discussed on the level of possibly apomorphic characters.

MATERIAL AND METHODS

The gastral exocrine glands of 34 species, belonging to 9 subfamilies were investigated. Live specimens were fixed in alcoholic Bouin and embedded in methyl methacrylate (Rathmayer 1962). The staining was Azan (Heidenhain).

RESULTS

Functional aspects

Functional-morphological studies on gastral exocrine glands reveal, that some glands probably produce lubricants, especially in those located at strongly sclerotized and moving parts of the integument resp. sting apparatus. The arguments in more detail:

1. Tergo-sternal glands are located at the strongly sclerotized proximo-lateral part of the tergites and sternites, where four gastral sclerites meet. These glands are present in almost all species with a quite strongly sclerotized integument (belonging to the subfamilies Ponerinae, Pseudomyrmecinae, Myrmecinae, Dorylinae, Ecitoninae). They are lacking or less developed in species with a relatively thin integument. [Myrmicinae, Dolichoderinae, Aneuretinae, (Formicinae)].

2. The ponerine ant *Pachycondyla tridentata* is characterized by a very thick and strongly sclerotized integument. In this ant big clusters of glandular cells were found dorsally, dorsolaterally, laterally, lateroventrally, and ventrally between almost each single segment (Jessen & Maschwitz 1983).

3. Glands associated with the sting apparatus (except the venom gland and Dufour's gland) are located at those regions, where the most intensive movements occur while stinging. This are the protrusion of the aculeus and of the lancets (Maschwitz & Kloft 1971). Secretion to the aculeus is supplied dorsally and laterally by the sting sheat gland and sting sheat base gland. While protruding the lancets the triangular plate moves counter clockwise. In these movements the secretion of the triangular plate gland probably serves as a lubricant for reducing frictional resistance.

Phylogenetical aspects

The genera *Dorylus* and *Aenictus* differ in some possible apomorphic characters: in *Aenictus* the 7th tergite is reduced, that means, the orifice of the pygidial gland's reservoir is transferred to the gaster's tip; in *Dorylus* the 7th tergite is not reduced. In *Dorylus* a well developed anal gland occurs which is lacking in *Aenictus* and in every other species investigated.

Common for *Aenictus* and *Eciton burchelli* is a reduction of the 7th tergite and a secondary chitinous layer of the 7th sternite to which a well developed glandular epithelium is associated with.

According to the glandular outfit and the modifications of the gastral segments associated with it, a monophyletic origin of *Dorylus* and *Aenictus* seems to be unlikely. A monophyletic origin of *Eciton* and *Aenictus* seems to be more likely, at least it cannot be excluded (see also Gotwald 1979).

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Morphological and Biochemical Differences in the Abdominal Glands of *Pheidole pallidula* (Myrmicinae)

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Different degrees of polymorphism exist in the widespread *Pheidole* genus. Extensive research has been conducted on glands producing pheromones or defensive compounds in dimorphic New World species, showing not only differences between neutral castes but also between species. Our study of the strictly dimorphic European species, *Pheidole pallidula*, contributes to the present knowledge of biochemical polymorphism in the genus *Pheidole*. By use of biometric, histological, chemical and ethological techniques, we have studied the abdominal glands (pygidial, Dufour's and poison glands) in the minors and the majors of *P. pallidula*.

MATERIAL AND METHODS

Abdomens of *P. pallidula* were fixed in gluteraldehyde osmium, imbedded in Spurr's resin and pygidial glands were observed on 2 μ m sections. Measurements were taken on freshly dissected glands. For chemical analysis, Dufour's and poison glands were dissected and extracted in n-pentane. Pentane glandular extracts were analysed by capillary gas chromatography and (Z,E)- α -farnesene identified by its ¹H N.M.R. spectrum.

Circular artificial trails with glandular extracts were used as bioassay for trail pheromone. Other pheromonal functions were investigated through preliminary experiments. All the abdominal glands were presented in random order on a slip of heavy paper (5 x 5 mm) laid 15 cm from the nest entrance. Reactions like threats, alarm runs, short bites to other workers, bites to paper and self-cleanings were recorded and counted within a circle (2cm diameter) centred on the paper, as well as the number of ants entering the circle.

RESULTS AND DISCUSSION

Pygidial glands are moderately developed in *P. pallidula* and the differences between minors and majors are small. The glandular reservoir is somewhat larger in the majors in which more gland cells were observed. This contrasts with the extreme development of this organ in *P. biconstricta* (Kugler, 1979).

Contrary to some New World *Pheidole* species (e.g. Wilson and Hölldobler, 1985 ; Law et al, 1965), the size's increase of the poison gland's reservoir between the two neutral castes is very moderate (volume ratio major/minor = 1.9 (n=61)) and skatole could not be detected in the chromatograms of majors' poison gland secretions. A marked trail-following behaviour is induced by circular trails drawn with the minors' poison gland and in a much lesser extent with the majors' poison gland extracts. The production of trail pheromone by the minors is consistent with their essential role in exploration and in the initiation of food and defensive recruitments.

Size dimorphism of the Dufour's gland in the two subcastes is remarkable (volume ratio major/minor = 11.2 (n=50)) and proportionally even more marked than the well known dimorphism of the head capsule (volume ratio major/minor = 7.6 (n=20)). This hypertrophy of the Dufour's gland in the majors is associated with a clear biochemical specialisation of the major's gland in the production of (Z,E)- α -farnesene, representing about 94 % of the compounds detected by GC. The farnesene is also present in the Dufour's gland of the minors but constitutes only 12 % of the compounds detected by GC. We estimate that the majors produce about 80 times more (Z,E)- α -farnesene than the minors. The biological meaning of this biochemical specialisation is not yet understood. The Dufour's gland of other myrmicine species is also specialised in the production of one or several farnesene derivatives but no biological function has been assigned to these compounds (e.g., Cavill et al, 1967 ; Cammaerts et al, 1981).

Our bioassays indicate that poison gland secretion of the minors attracts and alarms the ants. This reaction is probably caused by the trail pheromone. The Dufour's gland secretions of majors and especially of minors induce alarm runs and aggressive behaviours like threats and bites to other workers. Only weak reactions were observed in the presence of crushed pygidial glands of the minors but those of the majors induce bites to paper and threats as well as numerous self-cleanings. These preliminary results suggest that both Dufour's glands and pygidial glands could be involved in the defence-alarm reaction of the society but more information is needed before concluding that they are actually released during defence.

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The Fire Ant Sting Apparatus: A Case of Harmonious Parsimony

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The sting apparatus and related glands are of paramount importance to fire ant foraging efficiency and aggressive behavior, both of which also reflect some of the special adaptations of ants.

DEFENSE

Fire ant venom is composed primarily of 2-alkyl or alkenyl-6-methylpiperidine alkaloids that have a variety of physiological activities (antibacterial and fungicidal). The most cost effective utilization of both workers and venom in defense is to avoid direct confrontation by repelling adversary ants. When disturbed, fire ants can disperse venom through the air by raising and vibrating their gaster in a "headstand" posture. If the intruder is not repelled, the worker can directionally spray venom onto the intruder using another type of gaster vibration called the "aggressive waggle". Fire ants engaged in physical combat have the option of stinging or directly wiping venom on the intruder. Defensively, the fire ant has a hierarchy of behaviors ranging from passive repellent action to aggressive stinging. A corollary to gaster flagging behavior was observed in brood tending workers, who vibrate their gasters and deposit venom on the brood. This behavior helps combat the omnipresent microbes in the ant's subterranean habitat (see Obin and Vander Meer, 1985 for references).

TRAIL PHEROMONE

A trail of fire ants is the end product of the trail pheromone response, which is comprised of a hierarchy of behavioral and chemical sub-categories. The Dufour's gland (attached to the base of the sting apparatus) is the source of the fire ant trail pheromone, and its contents are deposited through the sting onto the substrate by workers returning from a food source. The trail pheromone can be divided into (1) recruitment, (2) orientation primer, and (3) orientation. Workers must first be recruited or attracted to the trail. This is readily demonstrated by the reaction of workers to a point source of Dufour's gland extract. Orientation along the trail is not the automatic next step, since the pheromone component responsible for orientation does not induce orientation in non-trailing workers. An orientation primer or modulator pheromone is required to induce orientation. In *Solenopsis invicta* these behavioral sub-categories of the trail pheromone are accompanied by distinct blends of chemical components from the Dufour's gland. In addition, large amounts of Dufour's gland extracts induce whole colony migration, and a disturbed individual can release Dufour's gland material to attract and alarm nearby workers (see Vander Meer, 1986).

QUEEN FUNCTION

Female sexuals also have a highly developed sting apparatus; however, they are not aggressive and will not sting even if harassed. Fire ant workers are attracted to their queen, who depends on them for grooming, feeding, and general maintenance. An olfactometer and surrogate queen bioassay was used

to determine that the attractive pheromone is stored in the poison sac and released through the sting apparatus. This accounts for the queen's well-developed poison sac and it allows her maximum flexibility in the release of its contents. The above bioassays were used to isolate and identify three chemicals responsible for the behaviors defined by the bioassays (see Glancey, 1986).

Investigation of the queen egg-laying mechanism for *S. invicta* revealed that the sting apparatus of the queen is intimately involved (Vander Meer, unpublished). The process begins with partial sting extension and is followed by full extension of the sting and opening of the oviduct. Wave-like abdominal contractions force an egg out of the oviduct and onto a track formed by the sting. The sting is then withdrawn across the egg. Chemical analyses have shown that material stored in the poison sac is deposited on the eggs during the egg-laying process. This can serve at least two functions: (1) The alkaloids in the queens poison sac have antimicrobial activity that can be passed on to the eggs. (2) The queen attractant pheromone, also found in the poison sac, attracts workers to the eggs ensuring them of prompt care (Vander Meer, unpublished). As with workers, there are a multiplicity of functions for the queen's sting apparatus and associated glands.

EVOLUTIONARY IMPLICATIONS

The order Hymenoptera is divided into several sub-orders based primarily on the morphology and function of the ovipositor. The ovipositor of Symphyta took the form of a rasp or saw, and in Parasitica the ovipositor sometimes takes on phenomenal shapes adapted to specific hosts. In both Symphyta and Parasitica the ovipositor is a conduit for egg deposition. In contrast the ovipositor of the sub-order Aculeata evolved into a sting and eggs pass through the female's vulva rather than the sting. The colleterial or accessory glands in other insects are associated with the ovipositor and function to increase in some way the fecundity of the deposited eggs; i.e., promote the adhesion of the eggs to themselves or to the substrate on which they are deposited. These glands were arbitrarily classified as the alkaline and acidic glands, and in Hymenoptera Aculeata they evolved into the Dufour's gland and poison gland, respectively. The above egg laying mechanism indicates that the accessory gland-ovipositor-egg interactions of the sub-orders Symphyta and Parasitica have not been lost in Aculeata, at least not in *S. invicta*.

Multifunctional roles for the exocrine glands associated with the sting apparatus have been reported for other ant species (see Vander Meer, 1983); however, none of them approach the parsimony associated with the fire ant sting apparatus. The multitude of different functions are harmoniously integrated into a finely tuned social insect system.

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Territorial Marking with Chemicals in *Atta sexdens rubropilosa*

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The use of pheromones to mark a general defended area has been recorded in laboratory colonies of *Myrmica rubra* (Cammaerts et al., 1977), *Oecophylla longinoda* (Hölldobler & Wilson, 1977) and *Atta cephalotes* (Jaffé et al., 1979). Vilela & Howse (1985) showed that leaf-cutting ants are able to distinguish their own territory, fighting against intruders on the basis of odour signals.

The following work was undertaken in an attempt to investigate the existence of territorial chemicals controlling this behaviour in *A. sexdens rubropilosa*.

The leaf-cutting ant colonies used for this study were laboratory colonies of *A. sexdens rubropilosa*, *A. sexdens sexdens* and *A. laevigata*. A sheet of white duplicating paper (21 x 15 cm) was placed on the nest table of the above colonies so that it could be marked naturally for 48 hours, and was then tested using the *A. sexdens rubropilosa* colony. Alternatively, identical sheets of paper were soaked with solutions consisted of extracts of the exocrine structures in the gaster of *A. sexdens rubropilosa* workers. Two extracts, each containing 8 glands or tissue samples, were made from the poison gland and a cluster of the other glands present in the tip of the abdomen: Dufour, sternal, valves and tergal glands. Each sheet of paper received 250 μ l of the extract, corresponding to half a gland, approximately, in 2 ml of ether. The papers after being dried were presented to the ants on one side of the food trail on the nest table. Five minutes later, their behaviour was recorded for each paper for a 10 minute period.

Workers of *A. sexdens rubropilosa* dip the tips of their gasters on the ground when exploring a new territory, in a similar way to *A. cephalotes*, as described by Jaffé et al. (1979). During this 'Abdomen Dipping Behaviour' workers, mark the area with a territorial pheromones. We also found that workers of *A. sexdens rubropilosa*, after entering a new area, hooked their abdomens in a peculiar way, which we called 'Abdomen Hooking Behaviour'.

These experiments show that workers of *A. sexdens rubropilosa* mark the area around their nests with a pheromone that is produced in the tip of their gasters, in a similar way to *A. cephalotes*, as demonstrated by Jaffé et al. (1979). These authors were able to conclude that the source of the pheromones in *A. cephalotes* workers is the valves gland. The components of this pheromone are as yet unknown. It is likely that the territorial pheromone of *A. sexdens rubropilosa* is also

produced by the valves glands, but we cannot exclude the possibility of secretions from the other glands in the tip of the abdomen, Dufour, sternal or tergal glands taking part in the marker pheromone. Indeed, it seems to us that (Z)-9-monadecene, a component of the Dufour's gland, may play a role in the marking process. This could be explained by at least one of the following hypotheses: a) since both Dufour's and valves glands are discharged through the sting, it is likely that a small amount of secretions of one contaminates the other, at least initially; b) it is also probable that the pheromone responsible for territorial marking is a mixture of secretions of these two glands. Our experiments are not able to further elucidate this phenomenon.

(Z)-9-nonadecene has been described by Bradshaw et al. (1986) is a pheromone component that regulates pickup, marking and transport of leaves in A. cephalotes. It seems to us that this compound may also participate in the process of territorial marking in A. sexdens rubropilosa.

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4.2 Intra- and Intercolony Communication

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Intercolony Communication and Recognition in Ants

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During the past two decades, considerable progress has been made in the analysis of communication behavior in ants. This investigation has been carried out chiefly on three levels: (1) the molecular basis of chemical communication (e.g., Blum 1981, Morgan 1984); (2) the behavioral mechanisms and underlying morphological structures employed in communication (e.g., Hölldobler 1978, 1982; (3) the behavioral ecology of communication systems in terms of adaptive significance (e.g. Oster and Wilson 1978, Hölldobler and Lumsden 1980). Many studies have focused on communication among colony members (trail-laying, alarm recruitment, etc.), while comparatively little attention has been given to communication between colonies.

Our recent work on territoriality and recognition between colonies of several ant species suggests that inter-colony communication is a major influence on the structure and organization of ant populations and communities. In this context the insect society may be considered as a single organism which, like any solitary territorial animal, "negotiates" territorial contracts with its neighbors by employing a repertory of behavioral interactions in which strength is displayed. Since the success of actual fighting depends largely on the sizes of colonies involved, it seems possible that they engage in strategies of information exchange to improve the outcome of their mutual assessment.

Inter-colony communication in *Myrmecocystus mimicus*

The honey ant, *M. mimicus*, conducts territorial tournaments, often involving hundreds of ants, in which almost no physical fights occur. Instead, individual ants engage each other in highly stereotyped aggressive displays. These tournaments defend spatiotemporal territories, and at the same time enable opposing colonies to assess one another's strength. Depending on the outcome of this assessment, the opponents either continue to fight a ritualized combat or shift the tournament area toward the weaker colony, thereby curtailing its foraging range. However, if one nest is considerably stronger than the other, it will escalate the contest into raiding and enslavement of the latter (Hölldobler 1976, 1982, 1983, Hölldobler and Lumsden 1980).

During the tournament, the ants walk on stilt legs while raising their gasters and heads. When two hostile workers meet, they initially turn to confront each other head on, then engage in a more prolonged lateral display in which the gaster is raised even higher and bent toward the opponent. After approximately 5-30 seconds, one ant usually yields and the pair separate, continuing to move on stilt legs. Each soon meets another opponent and the ceremony is repeated. If a nest-mate is encountered in this context, contact lasts only 1-2 seconds and is terminated by a brief jerking movement of the body.

The numerous threat displays between individual workers are integrated into a massive group display between opposing colonies. The groups' "strategic decision" to continue display fighting, to retreat, to recruit, or to launch an escalated attack depends on information transmitted between workers during tournaments, indicating relative strengths of their colonies. We have developed three models representing the most likely mechanisms involved in this inter-colony assessment: (1) estimating the proportion of nestmates versus opponents encountered at the tournament site ("head counting"); (2) sampling the sizes of opponents to assess the stage of development of their colony ("caste polling"); and (3) assessing local conditions (rather than cumulatively sampled encounters) such as the presence nearby of many opponents engaged in display fights ("queue flooding"). (See Lumsden and Hölldobler 1983 for details.)

A key feature in the functioning of tournaments in inter-colony communication is the ability of the ants to instantly recognize nestmates and discriminate conspecific foreigners. We have recently investigated mechanisms of inter-colony recognition in carpenter ants (Camponotus) which, like Myrmecocystus, form monogynous colonies and exhibit strong territorial behavior. Our preliminary results suggest that inter-colony recognition in these two genera is achieved by similar mechanisms, though it is becoming apparent that the recognition systems of different social insect species may be quite diverse (Gadagkar 1985, Carlin and Hölldobler 1986.)

Inter-colony recognition in Camponotus spp.

In a series of laboratory studies of several carpenter ant species we found that non-nestmates are recognized by means of phenotypic odor cues which may derive from several sources (Carlin and Hölldobler, 1983, 1986). Genetically-determined cues ("discriminators", Hölldobler and Michener 1980) are demonstrated by the relatively unaggressive response of sister workers, removed from their original colony as pupae & reared in separate groups, when encountering one another for the first time in a neutral arena. Workers reared under identical conditions, but originating from different colonies, interact with significantly more aggression. However, during approximately the first week following eclosion, workers also acquire, and learn to respond to, ambient recognition cues. If experimentally transferred into a foreign nest, adoptees identify sufficiently strongly with the characteristics of the foster colony to engage in lethal fighting with unfamiliar sisters, reared in their colony of origin.

Though acquired recognition cues can originate from food and/or nest material odors, these seem to be relatively unimportant in Camponotus. Genetically-determined cues may also be transferred among nestmates, forming a composite cue that may homogeneously represent all colony members (the "gestalt" model of Crozier and Dix 1979) or may be dominated by the discriminators of some individual(s). As the proportion of a group of workers adopted into a foster colony increases, the collective odor gestalt should come to resemble that of their original colony, and aggression toward sisters from the home nest should progressively decline. However, this does not occur; instead, the discriminators of the queen, acquired and learned by all colony members, appear to determine identification in interactions between colonies.

Groups of nearly 200 workers each, adopted to conspecific queens, prove equally aggressive toward all non-nestmates, whether kin or non-kin (Carlin and Hölldobler, unpublished). In addition, domination of the composite colony odor by the queen is correlated with her fertility (as measured by degree of insemination, ovarian development and brood productivity). Worker cues become more important if their adoptive queen is infertile, resulting in reduced aggression among non-nestmate sisters. This mechanistic association between queen dominance and fertility also suggests an evolutionary association between queen control of inter-colony recognition and of other aspects of colony regulation (Hölldobler 1984). For example, workers from a founding nest may desert their own colony and join a more dominant queen in some ant species, including Myrmecocystus mimicus (Bartz and Hölldobler 1982).

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SYMPOSIUM

Kin Recognition in Social Insects

Organizer: Dorothea Brückner

Multiple Inputs in the Nestmate Discrimination System of the Honey Bee

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Can honey bees use multiple cues in their nestmate discrimination system? Recent work has shown that individual worker honey bees produce cues that allow kin-group discrimination (Breed, 1983; Breed et al., 1985). Previous work, however, had shown that strong odors from the environment can modify the recognition characteristics of workers (Kalmus and Ribbands, 1952). In other social insects the nesting material (Gamboa, et al. 1986) or the queen (Carlin and Holldobler, 1986) play roles in the acquisition of recognition cues by individual workers. There is little knowledge of how cues from these diverse sources might be integrated by social insects.

I have initiated work which will 1.) test the relative importance of each of these possible inputs in determining the recognition characteristics of individual worker bees, 2.) examine whether cues from different sources remain distinct or blend, and 3.) determine, if the cues remain distinct, which cues are used in each behavioral context.

In this paper I describe results from experiments that initiate this line of research. These experiments first attempt to determine whether, in fact, the recognition characteristics of individual workers are affected by acquired cues. Second, the interaction between endogenously produced and acquired cues is measured.

MATERIALS AND METHODS

In these experiments the recognition characteristics of each individual was assessed by introducing it into a group of ten five day old workers that had been maintained in the laboratory. No such recipient group was used more than once, and all of the recipient groups received sugar candy with five percent pollen, by weight. The recipient group was scored as having accepted (engaged in cooperative behavior such as feeding) or rejected (bitten or attempted to sting) the introduced bees. In some cases the bees in the recipient group were sisters to the introduced bee; in other cases they were unrelated.

RESULTS

A strong "hive effect" was detected by marking bees and placing them into their natal hive. After 24 h they were removed and tested by introducing them into groups of sisters. 33 of the 37 bees (89%) tested were rejected. This is a significantly ($X^2 = 20.5$, $p < .05$) higher rate of rejection than that encountered by control bees maintained in the laboratory; 9 of 26 controls (35%) were rejected.

Preliminary exploration of the source of this hive effect indicates that exposure to empty comb may explain a large portion of the effect. Groups of ten bees were caged on empty comb obtained from

the natal hive. After 24 h of exposure to the comb 13 of the 20 bees (70%) were rejected by their sisters. Again, this is significantly ($\chi^2 = 4.6$, $p < .05$) different from the control level. Caging groups of ten bees over honey stored in the comb had the same effect (15 of 24 -- 63% rejected).

The interaction between endogenously produced cues and acquired cues was measured in a cross-fostering experiment. Bees were marked on emergence and returned either to their natal colony or to an unrelated colony. After five days they were recovered from the colonies and tested as described above. Control bees were maintained in groups of ten in the laboratory.

43 of the 90 (48%) control bees were rejected when introduced into groups of sisters; this establishes a baseline level for comparison. 69 of 90 (77%) of laboratory maintained bees were rejected by non-sisters; this is consistent with previous reports, and indicates an endogenous component in recognition.

80 of 90 (89%) of bees introduced into their natal colony and tested when five days old were rejected by their sisters. This is not significantly different from the rejection rate of bees placed in a non-natal colony; 82 of 89 (92%) were rejected.

DISCUSSION

These data indicate that in addition to the previously reported genetically correlated cues produced by individual workers (Getz and Smith, 1983; Breed, 1983) acquired cues play an important role in honey bee recognition system. These cues can, at least in part, be acquired from the wax of the combs; at present it is not known if the wax itself is a cue or if the wax serves as an intermediary for food odors or queen odors.

Perhaps the most interesting aspect of these results is that the cues acquired in the natal colony seem to be not at all correlated with those used by small groups of workers in the laboratory. In the testing context used for these experiments the acquired cues seem to override the effects of the endogenous cues.

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Discrimination of Kinship within Honey Bee (*Apis mellifera*) Colonies during Queen Rearing

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Kin selection has become established in evolutionary theory, providing a powerful framework to explain many behavioral phenomena confronting evolutionary biologists since Darwin. Due to its central role in this body of theory, testing of the assumption that animals can distinguish the degree of relatedness of other individuals is of central interest to social biology.

The polyandrous social hymenoptera pose an especially interesting case. The repeated evolution of eusocial behavior in Hymenoptera has provided some of the strongest support for the importance in kin selection, but the unusually high relatedness within Hymenopteran families assumes single mating, and it is now apparent that polyandry is common. Only if individuals are able to determine the degree of kinship and bias their investment accordingly would the argument still apply to these species. In addition, polyandrous social insect colonies provide a setting lacking indirect cues, such as location or frequency of encounter, reliably correlated with relatedness. For this reason, if they do show kin recognition, these insect societies are a likely place to look for the most complicated form of kin recognition: phenotype matching with oneself as the reference. Swarming seems the most meaningful context in which to look for kinship discrimination in honey bees, since here a individual queen, related to a given worker with a coefficient of .25 or .75, will inherit the enormous resources of the colony, and the rewards of biased investment are greatest.

Methods and Results

This research consisted of four closely related experiments. All shared a common assay of kin discrimination in queen rearing. I presented honey bees with a choice of brood of different degrees of relatedness to themselves, in pairs of queen cups, and recorded the number of instances in which the bees reared both, neither, or one or the other of the presented brood into queens. All transfers were reciprocal, so that paired brood from a pair of colonies was put into each of the colonies. The cells were introduced to queenright colonies, but in a region of the nest from which the queen was excluded. I compared the resulting numbers of "related only" and "unrelated only" pairs to a null hypothesis binomial distribution with $p=0.5$.

My first experiment used standard queen rearing techniques, giving the bees a choice between unrelated larvae and nestmate larvae (each a half sister to most of the colony's workers, and full sister to the bees of one patriline), transferred into cell cups containing royal jelly. Part I of Table 1 presents the results of this experiment. The bees reared nearly equal numbers of unrelated larvae and related nestmate larvae. This supports the null hypothesis of no preference ($p \approx 1$).

In order to control for the possibilities that the royal jelly masked odor differences, that kinship recognition only takes place during a narrow age window (as is known for diploid drone recognition), or that the presence of royal jelly signaled that the larva was already accepted, I performed the same experiment again, but transferred eggs (0-24 hours after laying) instead of larvae.

The results of this experiment were quite different. The bees showed a highly significant preference for the related eggs (Table 1 part II). Most of the selection of which eggs are reared actually seems to occur just after the eggs hatch, so apparently the bees select among very young larvae.

Table 1. Summary of results from reciprocal presentations of brood in 5 experiments.

Experiment Number	Choice Presented	Kin Reared	Non-kin Reared	Alpha Level
I	Nestmate vs. foreign-hive larvae	76	77	~1
II	Nestmate vs. foreign-hive eggs	87	57	0.015
IIIA	Unrelated vs. $G=.25$ foreign eggs	22	15	0.162
IIIB	Unrelated vs. $G=.22$ foreign eggs	27	14	0.030
IIIA+IIIB	Summed results from above two	49	29	0.015
IV	Related, novel vs. unrelated, familiar eggs	69	39	0.005

To eliminate the possibility that foreign eggs were discriminated against, not on the basis of genetic relatedness, but of environmentally-acquired "colony odor", I needed to unlink relatedness from colony odor in the eggs. I accomplished this by breeding bees to yield colonies in which the workers were related to each other, but which were separate colonies. Since colony odor is known to differ between hives due to differences in the food that each hive discovers, the colony odor of these related colonies would be different, but genetically based odors would be similar.

I used two different breeding schemes for the colonies in this experiment. In the first, I instrumentally inseminated queens with the semen from drones which were brothers. Within these hives, all workers were full sisters. Between related hives, workers were related to the same degree as half sisters ($G=.25$). There was no relatedness between bees from unrelated hives. In the second, I instrumentally mated queens with their brothers, and then allowed daughters of these queens to mate naturally, with many drones. Within the resulting colonies, most workers are half sisters, and few are full sisters. Between related colonies, workers are related with a coefficient of .22. I then offered these bees a choice between eggs from a related foreign hive ($G=.22$) and an unrelated hive ($G=0$).

The bees seem to base their discrimination on genetic cues (Table 1, part III); even when both come from foreign colonies, they choose the related over the non-related egg.

To distinguish bees somehow assessing the similarity of the larvae to themselves from the possibility that they are familiar with the genetic odor of larvae within their own colony, and simply reject novel-smelling larvae, I unlinked the relatedness of larvae and the bees' familiarity with the larvae. I confined queens on single frames, and reciprocally transferred the frames every 3 days between two colonies, so that all the larval brood in each colony was the offspring of foreign queen. Before the pupae emerged, they were transferred back, so that all the adults in each colony were the offspring of that colony's queen. Continuing this process, I established colonies in which the bees never experienced larvae which were related to themselves, but which were otherwise normal. Finally, I offered these bees a choice between eggs which were their sisters, but which they had not experienced, and eggs which were completely unrelated to themselves, but were like those which they had experienced. As shown in Table 1, part IV, bees preferentially reared the related larvae, even though they had never experienced such larvae, and had experienced the alternative larvae.

DISCUSSION

It seems that bees are able to make the fine discriminations of kinship of larvae which would enable them to discriminate among their full and half sisters when rearing future queens for the colony. The mechanism by which they do this seems to involve knowledge of their own phenotype, and comparison of available larvae with this model. This study provides some of the best evidence yet discovered for such a "self-referent phenotype matching mechanism". The principal significance of this study, however, is that it demonstrates the ability to discriminate kinship within a colony of mixed paternity. This ability is an important assumption in kin selection theory, and is important to our understanding of the evolution of social behavior in insects.

REFERENCES (and further detail) may be found in:

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Odor Cues in Honeybee Kin Recognition

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Olfaction has been widely implicated in conspecific recognition systems, including kin recognition in the honeybee *Apis mellifera*. Both acquired and inherited odors appear to provide recognition cues. Here we present results relating to the nature and origin of these cues. All experiments involve differential training of the proboscis extension reflex using a glass rod contaminated with various chemical. The training methodology is reported in detail in Getz, Brückner and Smith (in press) and is only outlined here for the sake of completeness.

MATERIALS AND METHODS

Clean glass rods were prepared fresh prior to each experiment by contaminating them in one of the following ways. They were either: rubbed on the upper thorax of individual adult workers; or rubbed around the inner surface of cells containing eggs, without touching the eggs themselves; or dipped into puddles of mandibular gland secretions that surround very young larvae; or pushed into the center of cells filled with a curled 5 day old larva, without visible injury to the larva; or used to extract unhatched eggs from cells using a drop of albumen placed at the tip of the rod as 'glue'.

In each experiment, approximately 30 bees were differentially conditioned to two different rods using an 8 trial training sequence immediately followed by an identical 8 trial evaluation sequence. Data were obtained by scoring the number of errors that an individual bee made during presentation of the second 8 trials. Each individual thus made from 0-8 errors and an error histogram and average error could be calculated for each group.

RESULTS

The following experiments were conducted using two rods that were: rubbed on the upper thorax of a different forager from the same hive; rubbed on the upper thorax of the same forager as a control for the above experiment; rubbed on the upper thorax of different newly eclosed workers; rubbed around the inner surface of cells as a control for the previous experiment; pushed into different cells containing 5 day old larvae; dipped into different cells containing puddles of mandibular gland (designated as 'food sources') secretions as a control for the previous experiment; used to attach different eggs from the same hive; used to attach different eggs from different hives.

The results are given in the figure below the references and can be summarized as follows. The 'Different Forager' and 'Different Eclosing Worker' results indicate that variation in chemicals associated with the cuticle wax on the upper thorax of individual workers is detectable by the workers. The difference between eclosing workers could not be explained by contaminants that are picked up in the cell and hence may be genetic in origin. To obtain more conclusive proof, however, would require an approach similar to that presented in Getz et al., (in press) relating to the heritability of volatile odors.

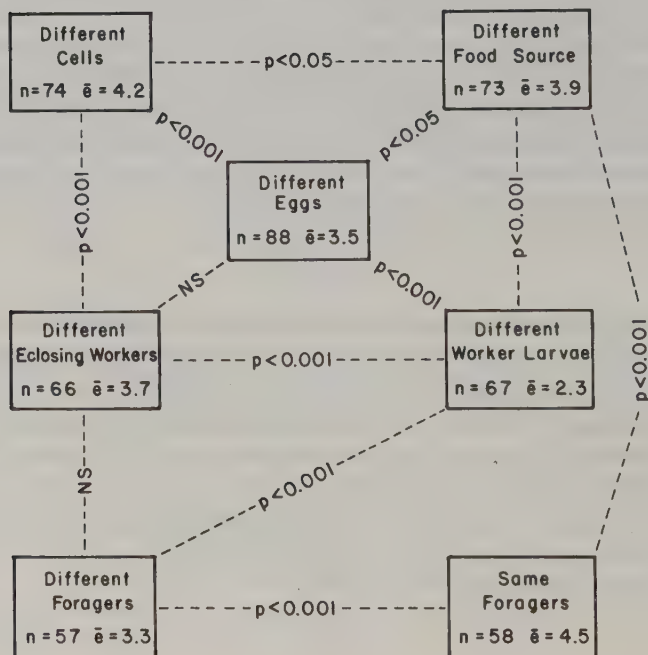
The strongest learning by far was obtained for different worker larvae. This result could be related to the fact that larvae in different physiological states (for example, degree of hunger) produce pheromones to communicate their state to nurse bees.

Finally, the results indicate that workers are able to discriminate between eggs irrespective of whether the eggs come from the same or from different hives.

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Significance of Results from Same Hive



Kin Discrimination of Brood and Young Workers in Two Races of African Honeybees

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Nestmate recognition has been demonstrated in a variety of social insects, yet the nature of recognition cues and the biological significance of discrimination remains unclear. Numerous studies have concluded that the recognition is genetically based and is in fact kin recognition (Getz and Smith 1983; Breed et al 1984). Other reports have emphasized the importance of the social environment and concluded that discrimination among kin is most commonly an effect of life history patterns (Carlin and Hölldobler 1983). Recognition would then be an effect; it is recognition by default entirely due to the fact that familiar individuals are likely to be kin. Recognition often appears both incidental and inaccurate. In honeybees, recognition and selection is most likely to act at the level of the larvae (Breed et al 1984) with special nutritional benefits being given to related larvae.

MATERIALS AND METHODS

In the summer of 1985/86 brood from *Apis mellifera scutellata*, *Apis mellifera capensis*, and hybrids of these two races, at different stages of development was marked with tippex and then either placed in a foreign colony or returned to the natal hive as a control. Sections of eggs, young larvae and late larvae were marked. Inter- and intra-race transfers were performed. The treatment of the brood was followed until cells were capped. All colonies used in this experiment were within 500 m of each other. For a second experiment, in the winter of 1986, colonies 30 km apart were used for transfers. This was intended to control for environmental recognition of larvae resulting from similar foraging activity of neighbouring colonies. The second experiment followed the same procedure as experiment 1, except that only *scutellata* colonies were used.

In addition, emergent workers from 3 colonies were marked and re-introduced into both natal and foreign colonies at 1 and 2 days old. Dead bee traps were placed at the entrance of the hives to catch all bees killed inside. Bee traps were checked daily for 3 days following the introduction and controls were run to test the efficiency of the dead bee traps.

RESULTS

The acceptance of all stages of brood by 'foreign' colonies did not differ significantly from the controls. Also inter-race transfers did not differ from intra-race transfers. The transferring of brood frames over large distances did not change the results and there was no increase in discrimination, but seasonal changes had a major effect on brood acceptance in both controls and foreign transfers. Foreign transfers are, if anything, more successful than controls in both experiments. Preliminary results indicate that 1 and 2 day old worker transfers did not result in discrimination against unrelated individuals.

Table 1. The percentage of normal development of brood in frames transferred between colonies of capensis, scutellata and hybrid colonies.

A. All colonies within 500 m of each other.

B. Colonies at least 30 km apart.

No of Transfers	% Normal Development		
	Eggs	Young larvae	Late larvae
A Controls n=1112	89.46	43.74	94.99
Foreign transfers n=2243	87.62	74.01	90.38
B Controls n=685	14.90	34.29	53.81
Foreign transfers n=1053	12.56	27.32	73.74

All results suggest that kin discrimination of brood in African honeybees does not operate and no active elimination of alien larvae was found. Normal development of all stages of brood was found to be high in both controls and foreign transfers. Previous studies have reported that social insects can recognize and discriminate against alien larvae, but most such studies involve royal larvae. While recognizing that it is the production of reproductives that is the central role kin recognition systems may play in social evolution (Page and Erickson 1984), it is a debatable point whether there would be a recognition system for larvae in a queen cell but not for larvae in workers cells.

It is a common bee-keeping practice to transfer brood between colonies, a practice which is predicated on the assumption that discrimination does not take place. The only class of brood that showed any significant level of brood removal was that of young larvae. The reduction in normal rearing in the second experiment is probably best explained by the change of season. The decreased temperature, and the reduction in brood production in southern African honeybees in winter is thought to be responsible for the high percentage of brood removal. Brood discrimination on the basis of kinship is absent in African honeybees.

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Using Group Metabolism as an Indicator of Kin Recognition in Honey Bees

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It has long been recognized that workers of honey bees (*Apis mellifera* L.) are able to recognize their own nestmates and kin, and distinguish them from members of foreign colonies. Behavioral studies suggest that environmental odors as well as genetically determined odor labels are used as recognition cues by individual workers.

Oxygen consumption measurements of groups of worker honey bees have been shown to be sensitive to small perturbations within groups including exposure to physiological levels of pheromones (Southwick and Moritz 1985). The sensitivity of the test system suggested that these metabolic measurements could be used to clarify mechanisms of kin recognition by highly volatile odors in the laboratory.

MATERIALS AND METHODS

Oxygen consumption measurements were taken of groups of 100 sister workers from one colony (test) exposed in an air flow-through system to the odor of another group of 100 sister workers from the same colony (control). After reaching resting metabolism each test group was exposed to the odor of a group of 100 sister workers (odor) from colonies with various degrees of relationship to the test group.

RESULTS AND DISCUSSION

Using this bioassay, we found a significant linear regression of metabolic reaction of the test group on relationship to the odor group. The results document that groups of workers in a collective reaction use highly volatile odor labels for discriminating related workers and drones from unrelated ones, and that these recognition cues are strongly genetically determined. The smaller the genetic relationship between the test group and the odor group, the larger is the metabolic activity of the test group to the volatile compounds of the odor group ($r^2 = 0.96$). The metabolic test also showed that workers are able to differentiate between brother drones and sister workers of the same colony. We also found that drones do not respond to workers or other drones from their own or unrelated colonies in this bioassay. Our results clearly demonstrate that workers can use volatile compounds in discerning related drones and workers. This study therefore strongly supports the hypothesis that odor cues are crucial for the identification of kin in honey bees.

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The Role of Kin Recognition in the Mating Preferences of Male Halictine Bees (*Hymenoptera: Halictidae*)

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The recognition of close kin in mating decisions would enable the avoidance of inbreeding, and may maintain optimal mating patterns with respect to maintenance of coadapted gene complexes. In the laboratory males of the Halictine bee *Lasioglossum zephyrum* can discriminate among genealogically related and unrelated females (Smith, 1983). Here we show that males of a second species, *L. malachurum*, make similar distinctions in a field setting.

MATERIALS AND METHODS

Female *L. malachurum* were collected from two different nesting aggregations in the vicinity of Tübingen in August and September, 1985. They were first killed by freezing, and then alternatively pinned in one of the two previously chosen test locations within the larger aggregation. The number of males attempting to copulate with the female was then counted for each minute during the three minute test. Afterwards, the female was removed and replaced in the same location two minutes later. To test the attractivity of females with different kinship to the males, either the same female, or a female collected from the same field nest, or a female from a different nest was then used. At all, 26 series consisting of these three tests were done. For to see if the males move throughout the entire aggregation, males were marked within predefined areas.

RESULTS

Marked males were never observed in other areas than those where they had been marked. Therefore, it can be assumed that mostly the same males were always tested within one location. In all the tests the mating attempt means for the first female tested decreased each minute over the three minute test. This decrease is probably due either to habituation or associative learning. Among the first bees tested, females from the foreign population were significantly more attractive to the males than females from the local one. When a female was replaced with herself, her attractiveness to the males was significantly lower than in the first test. When the second female tested was a sister of the first, fewer males were attracted than to the first, although the mean was higher than when the first female was retested. However, when the second female tested was a non-nestmate, then the number of males attracted to her was not significantly lower than to the first.

CONCLUSIONS

Therefore, males of two Halictine species obviously are able to discriminate females in subsequent matings. Foreign and non-nestmate females of the first mate are preferred. The evolution of the underlying recognition mechanisms which apparently make use of pheromonal cues was probably driven by avoidance of inbreeding.

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Nestmate Recognition in *Camponotus floridanus*: Behavioral and Chemical Evidence for the Role of Age and Social Experience

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The necessity of interactions with older nestmates during the very first hours of adult life to develop a normal ability to be recognized as a nestmate has been shown in *Camponotus vagus* and *C. floridanus* callow workers ants (Morel, 1983, 1986). Cross-fostering of callows between two colonies of *C. floridanus* demonstrated that young workers recognize as nestmates the sisters of their foster nurses, suggesting that recognition cues were transmitted from the foster nurses to the callows (Morel and Blum, 1986). The role of hydrocarbons present on the cuticle in nestmate recognition has been recently shown in *C. vagus* (Bonavita-Cougourdan and Clement, 1986). We report here the results of behavioral and chemical analyses that investigate the role of age and social experience on the ability to be recognized as a nestmate in *C. floridanus*.

MATERIALS AND METHODS

Two queenless colonies of *C. floridanus* were used for the bioassays. Foragers and callow workers (0 and 5 -days old) were tested. Callows were either nurse-raised (normal) or raised without any contact with older workers (naive). We measured the aggressive response of workers to individuals from one of the above categories introduced to either their own colony or to the alien colony. The introduced ant from all categories were presented either frozen, washed for 3 hours in hexane or washed and coated with a hexane rinse from a worker of the same category, either belonging to the same colony or to the alien colony. For each colony, 10 introductions of each category and of each treatment were performed. For each test the behavior of resident workers to the introduced ant was recorded every minute for a total of 10 minutes. Comparisons were made with the Mann-Whitney U test.

Four colonies with queen were used for chemical analysis. In two of them, the 5 worker categories were tested and for the other two, only foragers were used. The individual hexane-rinses, after concentration under nitrogen, were analysed by gas chromatography (DB1 30-meter capillary column, oven temperature from 50°C -1 min- to 285°C at 5°C / min). For each colony, 10 workers of each category were analysed. Twenty major peaks were selected. Comparisons with Newman-Keuls tests and principal component analysis were made on the relative proportions of these peaks. The bioassay described above was performed with live ants on the colonies used for chemical analysis. The same behavioral results were obtained on the two sets of colonies.

RESULTS AND CONCLUSIONS

Behavioral results. Dead ants from all categories induced more aggressive behaviors in the alien colony than in their own colony. However, there were differences between the 5 categories. Foragers induced the smallest number of

aggressive behaviors in their colony and the highest in the alien colony. In alien colonies, 0-day callows induced significantly lower aggressive behavior. In their own colony, naive callows induced significantly more aggressive behavior. In addition, 0-day naive callows induced less aggressive behavior in the alien colony than 0-day normal ones.

With washed ants, there is no difference between introductions in their own colony and in the alien colony (or the amount of aggressive behavior induced in the alien colony is considerably diminished), nor are there differences between the 5 categories of workers. Washed ants induced more aggressive behaviors in the alien colony when they were coated with rinses from their own colony, and the reverse when coated with rinses from the alien colony, except for the naive callows, which induced a high number of aggressive behaviors in their own colony.

Chemical results. TLC and column chromatography indicated that 85% of the hexane rinse was composed of hydrocarbons. Their carbon number ranged from 30 to 35.

Colonies were readily distinguished by the chemical profiles derived from any of the test groups. For example, the differences in the proportions of two forager peaks were sufficient to differentiate 4 colonies. The relative proportions of peaks also showed significant differences between test groups within the same colony. For each colony, 2 or 3 peaks (not the same ones) were sufficient to make the 5 categories distinguishable. Principal component analysis made on all the 5 categories of the 2 colonies also allowed separation of colonies and categories.

We conclude that chemical cues are involved in nestmate recognition in C. floridanus and these cues are modified as callow workers age. The very young callows specially, have a very different profile, which can be correlated with the lower aggressive response they induce in an alien colony. Social experience with older nestmates is necessary for callows to develop the colony specific profile needed for complete integration into the colony. In absence of interactions with older nestmates the naive callows develop a chemical profile very different from normal individuals of the same age, which can explain the aggressive behavior the naive callows induce in nestmates. These results were obtained either on queenless or queenright colonies, suggesting they are not queen-dependent.

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Nestmate Recognition and Cuticular Hydrocarbons in *Camponotus vagus* Scop.

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In *Camponotus vagus*, a European monogynous ant species, societies are "open" or "closed" according to the geographical distance between nests. Members of a colony are able to discriminate nestmates and alien individuals belonging to a distant colony experimentally introduced into their foraging area. The role of cuticular hydrocarbons in nestmate recognition in that ant was determined using both behavioural and chemical analyses (Bonavita-Cougourdan, Clément, 1986; Bonavita-Cougourdan, Clément, Lange). Chemical analyses and behavioural bioassays were conducted in 3 pairs of colonies exhibiting a strong aggressive behaviour between them.

CHEMICAL ANALYSES

Samples of cuticular hydrocarbons from heads, thoraces or both heads and thoraces and samples of post-pharyngeal gland hydrocarbons were extracted from foraging workers.

Combined G.C., T.L.C. and G.C./M.S. analyses indicated that samples were composed of saturated hydrocarbons only; 32 linear and branched saturated hydrocarbons (25 to 35 carbons) were the major components of the blend.

We found stable variations between head and thorax in same individuals. Relative proportions of the heaviest hydrocarbons are higher in the head than in the thorax.

The pattern of the cuticular hydrocarbon blend characterizes each society. Differences in relative proportions of hydrocarbons are much more important between agonistic couples of societies than between non agonistic ones. Between 2 colonies (A and B), for example, the greatest differences appeared for the following hydrocarbons: dimethylalkanes with 32 carbons (11,15diMeC30; 13,17diMeC30), with 33 carbons (5,11diMeC31; 5,17diMeC31; 5,19diMeC31), with 34 carbons (11,20diMeC32) and with 35 carbons (5,15diMeC33; 5,17diMeC33; 5,19diMeC33, 5,21diMeC33), - monomethylalkanes with 27 carbons (2MeC26) and with 32 carbons (7MeC31; 9MeC31), - and two n alkanes (nC25; nC27).

The postpharyngeal glands, previously described as being able to concentrate lipids from food (Janet, 1894; Darchen, 1976) and to synthesize hydrocarbons (Attygalle, Billen, Morgan, 1985), have been found to present the same hydrocarbon pattern as cuticular extracts. The relative proportions are closer to those found in the head than in the thorax.

ETHOLOGICAL ANALYSIS

Ethological tests consisted of various random introductions of either foraging workers or chemically treated surrogates or lures (10 tests of 15 min. for each category). Workers, alien or resident, were either alive or killed by freezing. Lures were made of dead resident

workers washed with organic solvents and covered with an extract of heads or thoraces, or heads and thoraces, of alien or resident ants. Behavioural aggressive responses of resident ants were divided into 2 types: type I: mandibular opening, nibbling, snatching up, snatching with traction, - type II: abdominal curving with venom spraying. The most aggressive responses are those of type II.

Resident workers dead (DR) or alive (AR) and lures without cuticular wax (W) or covered with extracts of their sisters (head (WRH) or thorax (WRT)), or head and thorax (WRHT) elicited no or very few aggressive responses, and only type I units.

On the other hand, introduction of alien workers, dead (DA) or alive (AA) or lures with cuticular hydrocarbon extracts of head (WAH) or thorax (WAT) or head and thorax (WAHT) from alien workers elicited a very strong behavioural aggressive response from resident workers. Table 1 shows the data concerning the two colonies A and B.

Workers or lures	AR	DR	W	WRH	WRT	WRHT	WAH	WAT	WAHT	DA	AA
Type I	-	0,4	2,4	1,6	1,3	2	42,6	37,6	48,1	71,2	106,9
units (m)	$\pm 0,37$	$\pm 1,27$	$\pm 0,76$	$\pm 0,76$	$\pm 0,59$	$\pm 5,94$	$\pm 5,51$	$\pm 7,44$	$\pm 17,14$	$\pm 21,03$	
Type II	-	-	-	-	-	-	3,8	2,3	3	6	7
units (m)							$\pm 0,30$	$\pm 0,89$	$\pm 1,30$	$\pm 1,30$	$\pm 3,18$

Tab. 1. Responses of resident workers against introduced worker or lure. m = mean (10 tests). The symbols are described in the text.

Crushed alien postpharyngeal glands or gland extracts of alien workers laid on a resident worker thorax determined, just as head or thorax extracts, a strong behavioural response from their sisters.

These results demonstrate that we can transfer to a worker the colony chemical odor, composed in *Camponotus vagus* of cuticular hydrocarbons. The relative proportions of some of them, especially dimethylalkanes, are responsible for the nestmate recognition.

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Sub-Caste Discrimination in the Ant *Camponotus vagus* Scop.

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In ants, members of a colony are able to discriminate nestmates and alien individuals belonging to other colonies of the same species. Nestmate recognition depends on chemical signals. We demonstrated that, in *Camponotus vagus*, the chemical signature of the colony is composed of cuticular hydrocarbons blent in proportions varying from one society to another (Bonavita-Cougourdan, Clément, Lange). Moreover, recognition signals are present within the society between members of various functional sub-castes: foragers are able to recognize brood-tenders and workers belonging to their own functional subcaste (Bonavita-Cougourdan, Clément, 1986).

Thirty ethological tests (10 in each society) were conducted on three queenright societies. When 5 brood-tenders (at least 5 (2 societies) to 7 (1 society) months old) and 5 foragers were simultaneously introduced into the foraging area, foragers brought back into the nest 76 % of the brood-tenders. They were carried with a *Formicinae*-typical posture or sometimes dragged. Introduced foragers, on the contrary, were never brought back.

To check the role of a possible chemical signal in sub-caste recognition, we extracted samples of cuticular wax of each functional sub-caste (brood-tenders carried out during the bioassaya and carrying foragers). It appears that the relative proportions of some hydrocarbons, especially light hydrocarbons (24 to 27 carbons) are significantly different between each functional sub-caste in the 3 colonies: light hydrocarbons are more abundant in foragers than in nurses. These variations could be correlated with the age of the workers: brood-tenders are generally considered to be younger than foragers.

Other chemical analyses showed that, between these two sub-castes investigated, there also appeared differences in hydrocarbons extracted from the postpharyngeal glands.

Conclusion: in ant societies, at least in *Camponotus vagus*, foragers are able to recognize brood-tenders. This discrimination could depend on differences in the relative proportions of cuticular hydrocarbons, possibly varying with age. Of course, other chemical or behavioural factors could also intervene in this discrimination.

Bonavita-Cougourdan A., Clément J.L., 1986. Processus de reconnaissance chez la fourmi *Camponotus vagus* Scop. Bull. int. S.F.E.C.A. In press

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Colony Recognition in the Ant *Cataglyphis cursor* (Hymenoptera, Formicidae)

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The closure of social groups is almost the universal rule amongst insect societies. It is based on colonial recognition as indicated by strong reactions towards non nestmates. The aim of this research was to investigate colonial recognition between adults which is known for many species as can be seen in other papers in this volume, but also colonial recognition of larvae by adults, which is less known.

Cataglyphis cursor was chosen because we observed in the field that during some periods of the year workers can pass from one nest to another. This is very exceptional in monogynous societies which are considered generally as very closed (1). All the experiments were carried out with colonies reared in the laboratory with the same food supply.

1. Recognition of adult nestmates

Workers marked with a dot of paint were introduced in the foraging arena of an alien society and observed for at least 2 days, after which they were generally adopted or killed. As could be predicted with field observations, when the tested colonies came from the same habitat, they accepted some alien individuals. The % of adoption varied a lot, from 25 to 90% (mean 54.5%). When the nests were very close to one another (less than 1 m) 90 to 100% of the workers were adopted (9). This exception to the rule of the closure of monogynous societies can be understood if we consider the mode of colony foundation in this species. The colony is founded by fission with a newly inseminated female (8). As a consequence in the same place colonies are more or less kin. We can suppose that relatedness between colonies decreases with the geographic distance as was suggested by adoption experiments. The % of adoption varies in negative correlation with the distance. In the extreme cases the adults intruders are almost always killed and macro-geographic variations can be identified in the species *C. cursor* (9).

It is well known with ants that callow workers are not considered as mature workers and they are always adopted when they are introduced into the nest chambers of any alien colony. This was verified in *C. cursor*. Otherwise in this species, when young ants are introduced in the foraging arena of another colony, they are sometimes recognized as aliens by the foragers. The 4 days old workers are always rejected and are considered as mature workers. This fact could indicate progressive change in the individual odour of the young ants. The adopted ants have been reintroduced at different ages in their native colony, where they were always recognized and accepted by their sisters (10). Finally these ants were accepted in 2 colonies where the mature workers do not tolerate each other. This fact is to be discussed with the problem of the origin of the colonial odour.

2. Brood recognition

Colony discrimination also exists for brood as indicated by differential brood-nursing activities on the part of adopted ants (7) or in the case of choice tests (5). It was observed that a familiarization with the odour of larvae can occur during the first days of adult life. If callow workers are transferred into an alien colony or reared in small groups with alien workers and larvae their preference for the sister brood is slightly attenuated, but not reversed (3, 5). So we hypothesized that colony-brood recognition could be acquired during preimaginal life.

To investigate this question, *Cataglyphis* eggs were transferred from their parent colony to a recipient colony. The larvae hatched from these eggs spent their whole larval life in an alien colony, and the cocoons were transferred back into their native colony. The adults were tested to detect their larval preferences. The workers showed a clear preference for the larvae of the familiar alien colony. This result was confirmed with larvae transfers at different ages. It was shown that the nursing behaviour of workers cannot be altered during the last instar of larval life. This demonstrates the existence of an **imprinting like learning**, occurring during the early larval life, a learning which determines the orientation of brood-nursing behaviours of the adult (5).

This research suggests that spatial proximity, or **fellowship** as proposed by Jaisson (6), between individuals is crucial for kin recognition which appears through a learning process. Under natural conditions colonial recognition is associated with genetic relatedness, and so fellowship is superposed on kinship. Altruistic behaviours are directed towards fellows, which are generally related individuals (2, 5). A genetic determination of kin recognition seems to be involved for some species but it is not a general rule (4). One can imagine that early learning is one way used by natural selection to assume the inclusive fitness of individuals, this means does not need genetic relatedness, and so this is a very flexible process.

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An Investigation of the Development of the Chemical Factors in Ants Intra-Society Recognition

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In ants, discrimination between sisters and intruders may be partly based on the identification of chemical signals which may be genetically determined substances acting on the insect's metabolism and/or derived from the physical and social environment (food, habitat, the presence of the queen...) (Errard, 1986). To effectively function as a colony member a young individual must be capable of both detecting and acquiring knowledge about such signals.

In this investigation we studied two phylogenetically distant species, *Manica rubida* (Myrmicinae) and *Formica selysi* (Formicinae) ($I = 0.20 \pm 0.05$, Nei's index) that live in the same biotope, in the Alps region. We used the experimental model of artificial mixed societies as an effective technique for testing the influence of early conditioning to a mixed colony odour upon interspecific recognition and modification of the species-specific cuticular hydrocarbons. The cuticular hydrocarbons were isolated by applying the concentrated wash to a silicic acid column and eluting with hexane or ether. The hydrocarbon fraction was analysed by gas chromatography (Jallon, 1986).

It was shown that there is a sensitive period for establishing preferential relations between individuals of these species. Thus, the association of *F. selysi* and *M. rubida* must take place within 24h of emergence if heterospecific interactions are not to induce aggression. Nevertheless, the investigation of the ontogeny of the appearance of cuticular hydrocarbons of these two species showed that each species produced qualitatively and quantitatively progressively more species-specific cuticular hydrocarbons during the 24 first hours after their emergence. It therefore seems plausible that the establishment of aggressive behaviours is correlated with the development of species-specific cuticular pheromones. In addition, when the two species were associated in artificial mixed colonies, each species acquired the cuticular hydrocarbons of the other. This accumulation of alien hydrocarbons continued up to 15 days by which time the cuticle contained higher molecular weight hydrocarbons. But *F. selysi*, which presented more non-agonistic inter-specific interactions than *M. rubida*, also acquired more alien hydrocarbons ($I = 0.87 \pm 0.06$) than *M. rubida* ($I = 0.55 \pm 0.09$). These data suggest a passive mechanism for the acquisition of alien hydrocarbons. Nevertheless, when the two species were separated, the analysis of cuticular hydrocarbons showed a decrease in alien related molecules with increased duration of separation. In the extreme case (after a 5-month separation), by which time, only the ants species-specific hydrocarbons remain, they are not treated as aliens when replaced in heterospecific groups, and the aggressions they suffer do not result in death. This would suggest the existence of a memory, used in the recognition of known individuals.

According to these results, young ants seem to be able to become familiarized with the first odours that they encounter.

Role of the Queen in Intra-Colonial Aggressivity and Nestmate Recognition in *Leptothorax lichtensteini* Ants

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In previous studies, I showed that in *Leptothorax lichtensteini*, the merging of two colonies involves several stages, the last of which, the elimination of one of the queens by the workers of both colonies, is necessary for the merge to take place.

Experiments were carried out on half-colonies in order to confirm that in this species the queen plays an important part in group closure mechanisms.

When a colony is split into two similarly composed groups which are kept apart for 4 months before being placed together again,

- if the queen has been left in one of the 2 groups during the 4-month period of separation, the two groups will remain separate and fighting will occur among workers in the area between the nests. The individuals in the 2 groups no longer seem to recognize each other.

- if the 2 groups are kept without the queen for 4 months, no aggressivity is manifested ; no merge occurs, however. The presence of the queen in one of the groups is thus necessary to nestmate recognition.

If the experimental set-up is rotated 180° so as to make the ants go towards the nest opposite their own, most of them flee when nearing the alien nest. This shows that not only the presence of the queen but other, nest-related factors are involved.

- if the queen is transferred from one nest to another every 2 weeks, the 2 half-colonies merge without displaying any aggressivity. This confirms that the queen plays a predominant role in closure and nestmate recognition mechanisms.

It should nevertheless be pointed out that the workers also participate, although seemingly to a lesser extent, in group closure : colonies which have been deprived of their queen even for quite long periods (4 months) are not systematically opened.

The following working hypothesis was formulated. In other species, cuticular hydrocarbons have been found to play a part in nestmate recognition mechanisms.

Chromatographic analyses are being conducted in order to determine whether cuticle substances from individuals isolated from their colony and individuals from colonies recently or less recently orphaned undergo any changes in the course of time. It is also proposed to investigate whether the chemical signatures of 2 half-colonies diverge in any way.

Individually-Produced Nestmate Recognition Cues and a Colony Odour "Gestalt" in Leptothoracine Ants

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Leptothorax ambiguus and L. longispinosus are closely-related species, occupy broadly overlapping ranges in eastern North America, and are extremely similar in various aspects of their biology. Colonies are facultatively polygynous, polydomous, and intercolonially aggressive within and between species. They are hosts for the same obligatory slave-making social parasites and are themselves occasional intra- and interspecific slave makers (Alloway et al. 1982).

Workers of these species, removed from their parental colonies as pupae and allowed to eclose and age for 36-69 days in social isolation, are readily accepted in their parental nests but are frequently attacked and killed in alien conspecific nests. In contrast, workers which eclose and age in allospecific nests are much less acceptable in their parental nests and may be attacked and killed. The response of parental colonies is an apparent function of the proportion of adoptees and original residents present in the mixed colonies. Mutual adoption between pairs of allospecific colonies results in a dramatic breakdown in aggression between the two nests: workers are readily exchanged and colony fusions are easily obtained. The presence of queens in paired mixed colonies has no apparent influence on this breakdown in aggression and, therefore, queens do not appear to be important proximate sources for nestmate recognition cues shared among adults as they are in certain Camponotus species (Carlin and Hölldobler, 1983).

These results indicate that individual colony members in these species produce and share nestmate recognition cues, and that nestmate acceptability is based on a collective (or "gestalt") nestmate recognition system. This type of system would provide the kind of inherent flexibility required in species which are polygynous, polydomous, and highly susceptible to social parasitism. Long postulated, this type of system has never before been clearly demonstrated. For a more complete account of this research see Stuart (1985).

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Kin Recognition, Polygamy and the Sex Ratio

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When workers on a single-foundress nest of an outbreeding and monoandrous haplodiploid social insect species control the sex investment ratio in the reproductive brood, the optimal (evolutionarily stable) proportion of males is predicted to be $1/4$ (Trivers and Hare, 1976). Inbreeding and local mate competition further increases the female bias while polyandry reduces it. If kin recognition is possible, optimal sex ratios are independent of the number of males a female mates with (Joshi and Gadagkar, 1985). The consequences when kin recognition is only partially possible are described here.

An infinite population with nonoverlapping generations is assumed. A single biallelic locus governs the sex investment ratio. The workers on the nest control the sex ratio of the reproductives. Kin recognition is modelled by the parameter k such that $k=0$ indicates complete absence of kin recognition (workers unable to distinguish between full and half sisters) and $k=1$ implies that investment in the females is confined only to the full sisters. Linearized difference equations are formulated to obtain changes in gene frequencies when an allele coding for an alternative sex ratio invades a pure population. Eigenanalysis of the associated matrix yields evolutionarily stable (uninvadable) sex ratios (Charnov, 1982).

RESULTS

When the gyne mates with N males and when the kin recognition parameter has the value k , the ESS proportion of males \hat{m} is given by

$$\hat{m} = \frac{1}{2} \left[\frac{N}{N+1} + \frac{N}{k(N-1)} - 1 \right]$$

If the number of matings per females is variable within the population, the harmonic mean of the number of matings should replace N in the above expression.

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Kin Recognition Cues: Their Complexity and Classification

(Basis of round table discussion)

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The costs to an animal of misidentifying kin are potentially high, because mistakes can result in lower reproductive success through the misdirection of resources during helping behavior, or in the suboptimal choice of a mate. Misidentifications may be either uninduced errors on the part of the recognizing individual, or they may be errors caused by the subversion of a recognition system by a conspecific or heterospecific cue bearer. One way in which the probability of making mistakes may be minimized is through the development of complex recognition cues that are more difficult for potential cheaters to mimic or to otherwise subvert. Naturally, the evolution of such signals involves the complementary development of highly discriminative sensory and neural mechanisms, but here I shall concentrate on the cues.

CUE COMPLEXITY

The simplest recognition cue imaginable would consist of a signal in one dimension of a single sensory mode, e.g., an auditory signal that is a monotone, or a chemical signal consisting of only one compound. A first order of complexity, carrying with it an increase in informational content, might then be generated by varying the amplitude of the tone or the concentration of the chemical, or by emitting either signal in simple or patterned pulses. A second order of complexity could result from the addition of only one more component to the signal in the same sensory mode, e.g., a second frequency or another chemical compound. If to this already complex signal were then added further components in the same sensory mode, and others in a different sensory mode, e.g., if visual components were added to the auditory signal, one would no doubt find that one's ability to grasp the extent of the complexity would quickly be exceeded. Yet many of the cues employed by animals in discriminating relationship enter this realm, especially at the level of individual recognition. It is small wonder, then, that although one may usefully model the genetics of recognition systems, e.g., the models of Crozier and Dix (1979) and Getz (1981), analyses of the recognition cues themselves presents a major challenge. A special difficulty resides in the fact that tests for recognition usually employ aggression versus a lack thereof, but a great many stimuli other than a mismatch of natural recognition cues can cause aggression, while lack of aggression is a negative. Hence investigators must devise sophisticated controls and be very conservative in their interpretations. Analysis of the cues involved in mating preferences, on the other hand, is somewhat less difficult.

CUE CLASSIFICATION

Hölldobler and Michener (1980) classified the chemical cues employed by social Hymenoptera in the discrimination of kin as either "dis-

criminator," i.e., recognition pheromones, or, when it was unclear whether the chemical cues were pheromones or substances of environmental origin, as "discriminating substances." I have retained the idea of dividing recognition cues according to their sources, but I have expanded the classification to accommodate cues in all sensory modes, so that the chemical cues of social insects may be placed in context with the recognition cues of other animals. Thus generalized, recognition cues may be of either intrinsic or extrinsic origin with respect to the cue bearer.

An intrinsic cue is a phenotypic expression of a cue bearer's genotype such that differences in a particular character, or characters, among conspecific animals reflect genotypic differences between them and these are used by discriminating individuals in the assessment of relatedness.

An extrinsic cue is a label acquired from the environment that serves to identify the bearer to a discriminating individual as kin on a probabilistic basis.

Intrinsic cues may be subdivided into three kinds according to the manner in which they are utilized by discriminating individuals. These are: 1. Cues of which no previous experience is required for reliable assessment of relatedness, such as visual cues used by pigtail macaques. 2. Cues that provide a basis for recognition through familiarity, e.g., sounds made by young bank swallows that are recognized by their parents. 3. Cues that provide a basis for class recognition via phenotype matching, such as the recognition pheromones of *Lasioglossum zephyrum*.

Extrinsic cues may be classified according to their sources. There appear to be four kinds: 1. Maternal labels acquired prenatally by recognized individuals, e.g., during embryonic development in tadpoles of the American toad, *Bufo americanus*. 2. Labels acquired postnatally from mothers or other close relatives, e.g., from the mother in goats. 3. Environmental cues that serve indirect recognition by location, such as a bird's nest or a wasp nest. 4. Cues acquired from environmental sources other than conspecifics, e.g., colony specific odors dependent upon diet in honey bees.

From this classification emerges something of special interest. While maternal and other transferred cues are extrinsic with respect to the cue bearer, they are mostly intrinsic to the donor, who is the discriminating individual, i.e., they are the donor's own extended phenotype (sensu Dawkins, 1982); even the probabilistic cues of category 3 are extended phenotypes. Thus, even the use of extrinsic cues in kin recognition leaves little to chance.

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Concluding Remarks

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The workshop on kin recognition was held to present and discuss all questions related to kin recognition in social insects; such as: Does kin recognition occur in ants, wasps and bees? Which is the biological context in which it occurs? Which signals do carry the information on kinship? The data presented in talks and posters did bear sufficient evidence to convince even the sceptics that kin recognition does occur in social insects.

Honeybees and ants provide excellent examples for the biological contexts in which kin recognition leads to specific behaviour; to mention only two such behaviours: Honeybee workers preferentially rear eggs of kin to queens even if the eggs are unknown to them. Ants exclude non-nestmates from the nest by severe aggressive behaviour.

The evolutionary strategies that are involved in increasing the inclusive fitness of the species that exhibit such behaviour are obvious.

The signals that are used in kin recognition are highly complex chemical signals composed of volatile and non-volatile components which are received via the antennae.

Chemical analyses of the components involved in kin recognition have yielded promising results. The recognition of complex patterns, for example those of the cuticular hydrocarbons, are an amazing neurophysiological accomplishment of the individual ant or bee.

Further exploration in the field of kin recognition will increase our understanding of the biology and evolution of social insect societies in particular and of biological systems in general. The experimental format of the workshop which consisted of 8 short talks, 6 posters and an extended round table discussion was successfully implemented.

4.3 Social Behaviour

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Foraging by Honeybee Colonies: A Case Study of Decentralized Control in Insect Societies

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INTRODUCTION

In 1971 Edward O. Wilson wrote that "the reconstruction of mass behavior from a knowledge of the behavior of single colony members is the central problem of insect sociology." This bold statement reflects two basic truths about the social insects. One is that their most remarkable achievements are mass phenomena: cooperative construction of properly proportioned nests, precise regulation of elevated temperatures inside nests, efficient performance of group foraging raids, and numerous other forms of coordinated mass action. The second fundamental fact is that at present we can only dimly perceive how the group phenomena of social insects emerge from the collective behaviors of a colony's members. In short, the social insects, especially the socially advanced species, present us with two distinct levels of description—individual and colony—but we have difficulty in bridging the two levels.

Perhaps the principal source of our difficulty here is that social insect colonies possess a type of internal control that is not familiar to us—they are organized around decentralized control rather than centralized control. We humans are accustomed to thinking about centralized control of groups, an organizational design in which there is a hierarchy of command and instructions pass from leaders down to others who actually do what needs to be done. Familiar examples of social groups with centralized control include business corporations, military units, and virtually all governments. Colonies of social insects, in contrast are organized around decentralized control. In a colony of ants, termites, social wasps, or social bees, there is no foreman who oversees a colony's operations and passes out instructions to the workers. Instead, each colony member forms her or his own independent decisions of what to do. The overall direction of a colony's activity at any one moment reflects hundreds or thousands of local decisions rather than a handful of global decisions. Thus the intelligence of a social insect colony is a collective intelligence, or, as Wilson (1984, p. 36) has so nicely expressed it, the brain of a social insect colony is the entire colony.

What is particularly puzzling about systems of decentralized control is how coordination is achieved, how it is that the many autonomous members of the group do what is good for the whole so that the whole operates as an efficient unit. Over the past 6 years my colleagues and I have worked toward solving this puzzle in the context of foraging by honeybee colonies. In particular, we have sought to understand how it is that the thousands of nectar foragers in a colony act and interact so that the colony as a whole stay focused on rich nectar sources despite constant changes in the locations of top-quality nectar sources. In this report I will first review our findings, then I will discuss several tentative generalizations about the organizational design of insect colonies that are suggested by our research on honeybees.

HOW COLONIES TRACK RICH SOURCES OF NECTAR

It is now clear from our studies that a colony's ability to choose among food sources is an automatic outcome of each individual forager in the colony following three basic rules:

- 1) if working a poor patch of flowers, abandon the patch,
- 2) to find a new patch of flowers, nearly always follow recruitment dances and only occasionally search on your own,
- 3) if working a rich patch of flowers, perform recruitment dances for the patch.

The net effect of these three rules being followed en masse is a steady migration of foragers away from poor patches, back to the nest where they follow recruitment dances, and finally out onto rich patches of flowers. Thus the question of how a colony chooses among food sources reduces to questions about how individual bees follow these three rules.

Detailed discussions of the behavioral programs behind these rules are found in Visscher and Seeley (1982), Seeley (1983, 1986), and Seeley and Levien (1987).

PRINCIPLES OF COLONY DESIGN

Let us now consider what generalizations about the organization and design of social insect colonies are suggested by this research.

1. The coordination of a colony's workers arises not through a hierarchial system of centralized control, but through a non-hierarchial system of decentralized control. For example, each honeybee forager decides for herself how she should respond to her particular patch of flowers based on her own perceptions of the patch's properties and of the colony's need for additional forage. But even without any one individual supervising the foraging operation, coordinated and efficient foraging by the whole colony emerges from the collective actions of the thousands of foragers in a colony.

2. The sophisticated achievements of a colony as a whole can reflect a small set of underlying rules of individual behavior. For example, it seems that the three rules of foraging behavior listed above are sufficient to account for the ability of colonies to choose among sources of nectar. Thus a certain simplicity can be hidden beneath the complexity of a colony's activities. However, this simplicity should not destroy our wonder about the achievements of whole colonies because the behavioral programs through which individual insects follow these rules probably will prove to be highly complex. This is certainly the case for foraging by honeybees. Thus once we identify the simple set of rules underlying a mass behavior, a new fascination is likely to arise about the intricate behavioral programs that enable individuals to follow these rules.

3. Feedback loops between individuals and the colony can be crucial to regulating a colony's activities. In other words, it is important to recognize that not only do behaviors of individuals shape the state of their colony, but also that conditions within their colony shape the behaviors of these individuals. For example, the foraging behaviors of individual honeybees determine their colony's rate of nectar intake, and reciprocally this colonial intake rate of nectar influences the foraging decisions of individuals.

4. Both external stimuli and a colony's internal configuration determine the behaviors of a colony's members. Thus the nectar foragers of a honeybee colony respond not only to external variables such as the properties of their flowers and the weather, but also to conditions inside the nest. Because of this responsiveness of individuals to conditions both outside and inside the nest, a colony as a whole is capable of both perception and self-perception. This remarkable fact explains much of the behavioral delicacy, self-involvedness, and capacity for cognition (Markl, 1985) that is so fascinating about colonies of social insects.

We are still a long way from understanding how colonies of insects translate the numerous behavioral acts of their members into higher-order effects. This challenge is not a trivial one because, as the generalizations just stated emphasize, colonies of social insects are composed of intricate parts integrated through complex interactions—they are truly "complex systems" (Simon, 1962). Furthermore, they are complex systems based on decentralized control, a form of group organization with which we have little familiarity. Nevertheless, I believe that the results of our studies of colonial foraging in honeybees should leave the reader with a sense of optimism, with the view that it is possible to make solid progress toward solving the "central problem of insect sociology."

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Distinguishing Features of the Social Behaviour of *Stenogastrinae* Wasps

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The knowledge of the biology of the *Stenogastrinae* has considerably improved over the last few years (Hansell, 1982, 1983; Hansell et al., 1982; Sakagami and Yamane, 1983; Turillazzi, 1985a; Turillazzi and Pardi, 1982; Yamane et al. 1983). The peculiarities of these wasps, however, which are distributed in the South East Asia Tropics, was appreciated by the very first researchers who saw them.

Here I will consider three aspects of the biology and social behaviour of the *Stenogastrinae*: brood rearing; interactions between nestmates and social organization in some of the species, and nest defence.

A strange milky substance, of a gelatinous consistence, found on the eggs and larvae of all the species so far examined, was at first thought to be food for the larvae. Interactions between adults and larvae are quite complex and complete, and adult females look after immature broods from the egg stage to the emergence of the imago. At the moment of deposition, the first drop of abdominal secretion the wasp collects is not used to glue the egg to the bottom of the cell, as previously supposed, but, in the genus *Parischnogaster* and *Liostenogaster* at least, the wasp holds it in her mouth and uses it to catch the egg as it emerges from the tip of her abdomen. The substance serves as a tool for collecting the egg and placing it inside the cell, where it is held in place by means of another secretion on its convex surface. After deposition, another drop of secretion is added to the first on the concave surface of the egg (Turillazzi, 1985b).

That the abdominal secretion does have a trophic function cannot be completely excluded, but if so it only represents a small part of the larval diet. From the very first days after hatching, the larvae are fed proteinaceous food such as drops of liquid regurgitated by the adults and small pieces of chewed insects. But the secretion has other important functions as well: contrary to other social wasps, *stenogastrine* larvae leave their egg shell completely and slide over the ball of secretion to position themselves on a plane perpendicular to the cell axis. The adults place the food on the secretion, where it is eaten little by little by the larvae. So the secretion serves as a microhabitat for the larvae, anchors it to the bottom of the cell, and acts as its cradle and its dish. The secretion is supplied to the larvae until they are large enough to stay inside the cells by themselves, by pressing their backs against the walls. The substance also functions as a food storage point for the entire colony (Turillazzi, 1985c).

Preliminary analysis by gas-chromatography showed that the chemical composition of the secretion is similar to that of the ant guards which some species of Parischnogaster and Eustenogaster use to defend their nests against ants. This leads to the conclusion that the two substances, very similar in appearance and consistence, are essentially the same and come from the same organ, the Dufour's gland.

Adults stimulate the larvae to make them open like a sphincter to receive food or fluid, and can also solicit them to emit drops of fluid (larva-adult trophallaxis). The adults usually hold their antennae apart and every now and then caress the body of the larva with them, while they stimulate the ventral part with their mouth-pieces. Such interactions are very frequent and are characterized by a slight back and forth movement of the body of the fluid-begging adult. During these visits, an adult may even collect food and fluid from one larva and take it to another larva or adult.

Adult-adult interactions are of various type and include begging for solid food, begging for fluid, aggression, and dominance interactions.

When foragers return to their nests, females and males run to meet them and try to grab the load of food with their mandibles. They start chewing the food trying to tear off pieces of bolus, with their heads against their partner's and making back and forth movements with their bodies and heads themselves. Antennal solicitation by the begger wasp on the forager is very rare, while the use of the anterior legs to grasp the bolus of food is quite common.

Fluid request is somewhat different. The begger wasp antennates its partner calmly, delivering light blows on the side of her head near the antennal sockets but biting her vigorously on the inferior part of her clypeus and mouth parts, and caressing the sides of her head with the anterior legs. The solicited wasp stops with her antennae apart and emits a drop of fluid which is sucked up completely or in part by the begger wasp. Fluid request can be observed even when a forager has not recently come back to the nest. Sometimes wasps that have just taken fluid from a larva are solicited to regurgitate fluid by their nestmates.

Dominant individuals usually crawl round their nests quite sure of themselves and move towards other individuals they meet in their tracks. On such occasions, subordinates may try to avoid the dominants or immobilize themselves by freezing on the spot. Then they assume a position with their head turned to one side and after the dominant wasp antennates them briefly, emit a drop of fluid between their mandibles. However the dominant does not usually suck up the fluid but passes by. In this case, the regurgitation of fluid has an obvious ritualized meaning. The function of such behaviour, similar to that in other social wasps, is clearly to placate the aggressiveness of the dominant female.

Aggressive behaviour towards nestmates is however rather rare. Dominant females pursue other individuals all round their nests, trying to bite them and hit them with the abdomen. This type of behaviour is especially directed

towards the males in an attempt to get rid of them from the colony.

Fights can easily be induced by dangling a partially immobilized conspecific from another colony, tied to a stick with a hair or a nylon thread, in front of a nest. The owners, after antennation and abdomen bending (a sure sign of aggressiveness), attack the intruders with abdominal blows, grasp them and fall from the nest together.

In some species, observations of nestmate interactions has led to the individuation of dominance hierarchies. In P. nigricans an essentially linear hierarchy of the Polistes gallicus type has been reported (Turillazzi & Pardi, 1982). This is essentially the same as Yoshikawa et al. (1969) observed in a colony of Parischnogaster probably of the jacobsoni group. In these species ovarian development and division of labour between the females are in relation to their hierarchical status.

Early authors had already noted the absence of aggressivity and the very shy nature of these wasps. The principal tactics in nest defence are the camouflaged nests and the choice of nidification sites where their main predators (ants and hornets belonging to the genus Vespa) are practically absent. Nests and colonies are always small in size and nest architecture obeys essentially three characteristics: mimicry, sturdy construction material and defendability against predators such as ants. Sometimes the nests are camouflaged so well that the best defensive behaviour the adults can take against predators which prey on sight is to remain motionless or to fly away. In some species the wasps fall from the nest at the slightest vibration of the substratum and start flying just a few centimeters below. Perhaps this behaviour makes it even more difficult for the predator to detect the position of the nest.

In contrast to the shy attitudes exhibited in presence of larger predators, females are constantly on guard against extraneous conspecifics which fly too near the nest, and will attack them in flight. They hit the approaching enemy in mid air, forcing them to fly away. This kind of defence is typical of species of Liostenogaster, Holischnogaster, Metischnogaster and Parischnogaster, but I have never observed it in species such as Parischnogaster alternata, which usually nest in very dark places.

Defence against ants includes the construction of sticky barriers placed on the substratum of the nest (Turillazzi and Pardi, 1981). However, the adults also actively defend their nests against scout ants. In Metischnogaster I twice observed a very interesting behaviour against a scout ant. The foundress of a three-celled nest, after an unsuccessful attempt at eliminating the ant, abandoned the nest only to suddenly land on it again, making it swing violently. This oscillation, in one case, was sufficient to make the ant fall off the nest.

Stenogastrinae present not only morphological but also important biological and behavioural differences to other social wasps. Biological and behavioural characteristics such as the progressive nutrition of the larvae,

larvae-adult trophallaxis, the extraction of the larval peritrophica, social dominance and division of labour, must be seen as evolutive convergences and/or parallelisms reached independently. The reduced colonial size, the camouflaged nests and the special methods they use to collect prey can be considered as adaptations to the particular forest environment and predation by ants and hornets.

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SYMPOSIUM

Foraging Strategies

Organizer: James F.A. Traniello

Introduction

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The behavior and ecology of foraging are among the most intensively studied aspects of social insect biology. The reason for this great interest is easily understood: social insects play a critical role in the trophic structure of temperate and tropical ecosystems, and are important predators, herbivores, pollinators, and decomposers. In terms of diversity and biomass, social insects comprise a principal faunal component.

A colony of social insects can have perhaps millions of foragers that may do little more outside of the nest during their lifetime than search for and harvest food. Therefore, analyzing the foraging strategies of social insects is simplified because sterile foragers do not have to partition their time and energy budgets due to the conflicting demands of mating, yet made more complex because both individual and group effort - and their interaction - must be considered. To maximize food intake and therefore reproductive success, a colony must sample its resource environment and select the most profitable resources. This selection process involves the efforts of individual workers and, through communication, the collective action of groups of foragers. Components of foraging strategy, which may include caste, communication, search behavior, learning, and all decision - making processes that underscore foraging can perhaps best be understood through an analysis of their regulatory mechanisms. Energetics and ecology are important reference points.

In view of the wealth of natural history information on their foraging habits, the abundance and diversity of species, and the relative ease with which they can be studied, the social insects provide a number of excellent model systems for the study of the evolution, ecology, and control of foraging. It is the purpose of this symposium to present current trends in research on social insect foraging behavior and update findings in ongoing areas of investigation.

The papers that follow are by no means representative of all current interest in the study of the behavioral ecology or sociobiology of foraging. Rather, several areas of research are offered as a sample of the wide range of projects being conducted. Paul Schmid-Hempel and Keith Waddington address two different problems in honey bee foraging, one involving the decision-making mechanisms of individual bees and the other, factors contributing to the accuracy of communication involved in the allocation of groups of foragers to resource patches. Schmid-Hempel presents economic models that make predictions about when honey bee foragers should stop collecting nectar and return to the hive, and experimentally tests hypothetical rules that foragers may use to implement such a decision. Waddington considers the role of body size on the effectiveness of recruitment communication and shows how workers of dif-

ferent sizes have different intake rates of nectar and therefore perceive different resource profitabilities, resulting in an inaccuracy in the information communicated during the organization of cooperative foraging. From a different aspect of energetics, Mogens Nielsen's laboring analysis of oxygen consumption in ants is extended to measure the cost of food transport. At the community level, Steve Rissing and Cesare Baroni-Urbani contrast the foraging strategies of sympatric seed-harvester ants to examine how different foraging systems may lead to differential resource use and favor species coexistence. Rissing also presents evidence of an intriguing annual cycle of body size fluctuation in Veromessor pergandei, and offers several hypotheses and tests of its significance. Finally, both Jacques Pasteels and his collaborators and I deal with the relationship between individual behavior and collective action, from both theoretical and empirical perspectives. I attempt to show how prey profitability and competition affect the decisions of Formica schaufussi workers to forage solitarily or induce social retrieval, and how search strategy is influenced by patterns of resource distribution. Pasteels and his coworkers, using a mathematical model applied to foraging in Tetramorium caespitum, describe how emergent, collective activity can result from identical, randomly behaving workers organized by non-linear communicative processes.

Again, this symposium only touches upon a few facets of social insect foraging. Regrettably, studies on social wasps and termites are almost entirely lacking. We hope the proceedings of this portion of the congress will be valuable in stimulating research on these groups, as well as extending research on ants and bees.

Foraging Strategies of Honeybees: Economics, Rules, and Fitness

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Workers of the honeybee (*Apis mellifera*) collect large amounts of pollen and nectar for maintenance and growth of the colony (Seeley 1985). It is likely that the strategies used will have effects on growth rate of the colony and thus on reproductive success. What strategies, then, do individual bees use, and what would the consequences for fitness associated with different strategies be? Earlier studies (e.g. Schmid-Hempel et al., 1985) focused on the crop-filling behaviour of worker bees, and showed that this behaviour could not be predicted by a strategy that maximizes net rate of energy delivery to the hive (e.g. Orians and Pearson, 1979). Instead maximization of net energetic efficiency (net gain per unit of energy spent) was closer to the observed behaviour. I report on field experiments that extend these findings to situations where various parameters of nectar availability vary simultaneously.

FORAGING STRATEGY

Individual bees were trained and tested in patches of artificial flowers. The flowers offered a constant reward at each visit, thus nectar availability did not diminish with time. Conditions were varied such that each individual experienced a single parameter combination of: patch distance (40 and 400 m from the hive), density of flowers (high and low), volume (0.7 and 1.3 μ l) and concentration (30%, 40%, and 50% weight per weight sugar solution) of nectar. Crop loads, as measured by the number of rewards taken, were observed and compared to the predictions of the two models mentioned above. A total of 24 individuals were tested. Observed and predicted crop loads differed significantly for the rate model ($P < 0.0001$). They were not different from and correlated with one another for the efficiency model ($r = 0.544, P < 0.0001$). Nectar volume and concentration, as well as flower density contributed most to variations in foraging behaviour of the bees.

RULES-OF-THUMB

Earlier authors suggested that bees might stop collecting nectar and return home to unload when

either a critical load weight or crop volume is reached (e.g. Kalmus, 1939, Rau, 1970). I tested the predictions of these two simple rules by manipulating the weight gain of bees during nectar collection. Extra weights were put onto the thorax and removed when the animal returned to the hive (this removal is crucial to the options available to the forager). Three treatments were compared: (1) Added: extra weight stepwise increased (up to 35 mg). (2) Removed: a full load of 35 mg at start of patch stepwise reduced to zero, and (3) Control. In all cases, nectar load (in crop) was increasing monotonically. The bees returned with the same crop load under Added and Removed, but earlier than the controls did. Therefore, neither of the two simple rules is supported. Rather, it seems that bees use a rule that includes the "effort" made in carrying loads throughout the patch visit.

CONSEQUENCES FOR FITNESS

Theoretical studies (Maczevicz and Oster, 1976, Houston et al. in prep.) show that the best policy for a colony of social insects is to divide its cycle into an ergonomic stage where only workers are produced, and a reproductive stage where only reproductive forms emerge. During the former, the growth of the colony should be at maximum. Growth rate is given by rate of production of new workers (and thus by rate of resource collection) minus rate of mortality. If mortality is foraging-dependent, then more conservative strategies than maximization of net rate will result in maximum reproductive output. This theoretical requirement may explain the nectar-collecting strategy actually found.

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Foraging Behavior of Honeybees

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Foraging is a complex behavior for which many of an animal's facilities come to bare. Information is received and processed, and decisions and responses are made. The purpose of this paper is to review a line of inquiry that attempts to understand and predict some aspects of honeybee behavior of foraging for nectar based on the energetics of foraging. Three questions are addressed: 1) what is a foraging bee's schedule of actual costs and intakes? 2) what is the nature of perceptions of these costs and intakes? 3) how is information on costs and intakes used to make foraging decisions?

ACTUAL COSTS AND INTAKES

Nectar is the bees' primary source of energy. It consists primarily of sugars and water. The concentration of sugars in nectar and the volume of nectar per flower varies greatly within and between species of plants.

Time and energy are spent during all foraging activities. Flight costs a bee weighing 0.1 g about 0.67 calories per minute (metabolic rate is 80-85 ml O₂/g body weight/h and 1 ml of O₂ consumed is equivalent to about 5.0 calories expended). Energy expended to handle flowers at low air temperatures approximates flight-expenditures. However, at 30°C a 0.1 g bee expends just 0.1 calorie per min. Flight time and energy spent in flight during foraging are a function of distance to the floral patch, velocity of the wind, and distribution of plants and of flowers on plants. Handling costs are affected by floral morphology and the quantity and concentration of nectar. In addition to time and energy costs, there are other costs. Foraging is strenuous and results in wear and tear on the bee's structural and biochemical machinery. Neukirch (1982) demonstrated that once foraging begins a bee has a fixed total flight performance of about 800 km. Foragers are also subject to considerable risk of predation and accidental death.

PERCEPTION OF COSTS AND INTAKES

The honeybee's dance reflects the dancer's perception of its experience in the field. Frisch (1967) and his colleagues studied perception of "profitability" using the waggle dance. Several aspects of the waggle dance vary with changes in costs and intakes. Waddington (1982) found that the rate that sharp turns of the round dance were performed (called RATE) varies inversely with the costs to fly to a flower to suck sugar solution and directly with the caloric intake per visit.

Foraging models assume that costs and intakes are additive along the same scale. Waddington (1985) tested this assumption. Costs and

intakes were varied for a bee to forage between two artificial flowers. RATE of the round dance was "read" inside the hive. A stepwise multiple regression analysis was used to create a "surface" of the perception of costs and intakes (RATE was the dependent variable). Fifteen bees were tested singly at the floral patch. The assumption that assessments of costs and intakes are additive on the same scale is met if the regression coefficients for the two energy variables have the same absolute magnitudes. There were three significant findings of this study. 1) Costs were weighted in relation to intakes. The mean of the absolute values of the regression coefficients for cost was five times greater than the coefficients for intake. Schmid-Hempel et al. (1985) also found that bees weight costs in deciding when to stop foraging and return home. 2) Dance behavior differed among individuals. 3) The bees' assessments of cost were more variable than their assessments of intakes.

FORAGING DECISIONS

The cost-benefit approach has proven useful for studying choice of flowers and flight path between flowers. Several investigators have found that honeybees preferentially visit flowers with lower costs and higher intakes. However, the details of the selection process have not been worked out. For example, it is not known whether costs are weighted when choosing. The distance flown between flowers in the field is affected by nectar volume in previously visited flowers. The directionality of inter-flower flights is affected by handling costs but may not be affected by caloric content of flowers.

CONCLUSION

To date, field observations (e.g., "flower-constancy"), results of experiments using artificial flowers (i.e., mechanisms of foraging), and the utility of evolutionary models have been viewed separately. Future work should be aimed at joining these techniques and results to more fully understand honeybee foraging behavior.

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Energetic Cost of Foraging

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One of the major disadvantages of social behaviour is that food has to be transported back from the surroundings, sometimes over long distances, to the nest or hive, and, therefore, a high proportion of the worker population is occupied with transport of food. Consequently, a substantial part of the energy flow through the society is used for transport. In general, bigger societies have to find their food from longer distances away, and they need therefore a higher proportion of workers for transport than do smaller societies. Thus, transportation of food can be a limiting factor for colony size.

MEASUREMENT OF THE COST OF TRANSPORT

In order to investigate the cost of transport, it is necessary to measure the loss of energy through respiration under the influence of various factors.

The first step in the series of necessary respiratory measurements is to define and measure the "basal metabolism" or resting respiratory rate. For many insects it is very difficult to prevent muscular activity, and therefore Holm-Jensen *et al.* (1980) anaesthetized ants in an attempt to establish suitable standard conditions for "basal metabolism".

Jensen *et al.* (1980) investigated the cost of running and Nielsen *et al.* (1982) studied the effect of load carriage on the respiratory metabolism of running worker ants of *Camponotus herculeanus* (L.). The cost of running was determined using a small threadmill where the amount of liberated CO₂ could be determined after the ants had run a certain distance. In order to measure the effect of loaded running, small platinum loads were glued to the thorax of the ants before they ran in the threadmill. From this series of experiments, it was possible to calculate the "resting metabolism" (ul O₂/mg/hour or J/mg/hour), the cost of running (J/mg/km), and the extra cost of carrying load (J/mg/km). A full description of the energetic cost of foraging for a society require measuring of the effect of temperature on these parameters and combine them with the relevant field observations.

The respiratory rate of bees in free flight has been investigated by Heinrich (1980) who found values of 80-85 ml O₂/mg/hour. Seeley (1985) used these values to make calculations of the energetics of foraging of honeybees.

The conditions for flying insects are quite different from running insects, primary because flying is extremely costly compared with resting (and running). The specific respiratory rate for honeybees during flight is 80 times higher than for *Camponotus* workers during running, but the cost of transport (energy expenditure/weight/km) for the bees is four times smaller. The reason for this difference is the tremendous difference in speed; honeybees fly approximately 25-30 km/hour whereas *Camponotus* workers run only 75m/hour.

DISCUSSION

The main source of error in the calculation of the energetic cost of foraging is the necessity use of data from artificial laboratory experiments and subsequent application of these to field conditions.

One of the factors whose effect is best described is temperature, and it is normally easy to make a correlation with respiratory rates and temperature. However, many phenomena related to temperature are not taken into consideration and therefore the results obtained can be quite misleading.

The temperature of the animals can differ very drastically from the temperature of the surroundings, e.g. ants can be up to 10°C warmer than the surroundings due to absorption of heat, and conversely, "cold ants" from the nest can forage for a short period in very warm surroundings before they are heated up. Before insects can fly, the temperature of the thorax must be quite high, and often higher than the surroundings. For honeybees, the thorax temperature must be above 27°C, and they have a very powerful thermo-regulation, which allows the bees to forage in the ambient temperature range 5°C to over 45°C.

Acclimatization is another factor where the effects are unpredictable. Nielsen (1986) studied the respiratory rates of ants from different climatic areas and found that the ants reacted differently to temperature changes, and that the respiratory rate also depend of the time of the year.

The number of individuals within the respiration chamber can alter the respiratory rate by a factor of two, and therefore, comparison of results from different investigations is difficult, because the number of individuals in the respiration chamber is generally selected according to the sensitivity of the equipment.

Besides the above mentioned factors, several others can be added, and their effects have been only sparsely investigated, e.g. humidity, altitude, body composition and light intensity.

Before a detailed description of the energetics of foraging can be achieved, far more studies have to be carried out, and new and untraditional methods have to be developed, in order to support and verify the traditional methods.

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Foraging Strategies, Diets, and Reproductive Behavior of Two Sympatric North American Desert Seed-harvester Ants

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The seed-harvester ants Veromessor pergandei and Pogonomyrmex rugosus are abundant throughout much of the xeric United States; they overlap extensively at the interface of the Mohave (where V. pergandei prevails) and Sonoran (where P. rugosus prevails) Deserts (Creighton 1950). Here I summarize research to compare these ecologically similar species and to understand possible mechanisms permitting their co-existence.

MATERIALS AND METHODS

Colonies of both species were studied at several sites in and near Phoenix, Arizona during the last 5 years. I have monitored worker body size and diets of 4 colonies of both species at the Desert Botanical Garden and South Mountain Park (both in Phoenix, AZ) for 3 years by taking monthly samples of foragers and any seeds they carried. Reproductive activities of both species have been observed throughout this time.

RESULTS

Veromessor pergandei and Pogonomyrmex rugosus have similar diets at both sites: at the Botanical Garden both ants harvest exclusively fruits of the grass Schismus arabicus and only harvest other seeds (almost exclusively those of the annual Plantago insularis) in Fall and Winter or during periods of low seed production, e.g. a drought in 1984. Similarly, at South Mountain Park both species take the above two seeds and rank a third species, Pectocarya recurvata, intermediately (Rissing, unpub. data). Unlike diet, the ants diverge with respect to patterns of worker size variance: V. pergandei colonies display a distinct annual cycle in worker size, explaining up to 65% of overall variance in body size, that is not found in P. rugosus (Rissing, in press).

The ants also diverge with respect to colony foundation and other aspects of reproduction. Mating season of V. pergandei occurs during the winter, and mating appears very localized; colonies are often pleometrotic (Pollock and Rissing 1985). Mating season of P. rugosus occurs during the summer and includes massive area-wide "leks"; colonies are almost always haplometrotic (Hölldobler 1976).

DISCUSSION

Both V. pergandei and P. rugosus are group foragers, have similar diets and forage at similar temperatures. The species diverge, however, in their reproductive ecology and behavior. Starting colonies of V. pergandei are clumped in the bottom of sandy

ravines where they are apparently closer to ground water. Adult colonies, on the other hand, are territorial and fight whenever their foraging columns overlap. Given this territoriality, starting colonies are under fierce territorial competition. This competition takes the form of reciprocal brood raiding soon after colonies open. Pleometrosis permits more rapid production of a larger worker force, and pleometrotic V. pergandei colonies prevail over haplometrotic ones in laboratory trials (Rissing and Pollock, in press). The primitive "polymorphism" of V. pergandei may impart a similar advantage to adult colonies. By reducing worker size during periods of resource depletion, a large and constant worker force can be maintained. Resources for worker production are likely most limited during Winter when preferred seeds are less available, foraging periods are limited and alates are likely produced. Territoriality leads to direct worker conflict (Went et al. 1972, Wheeler and Rissing 1975), and a larger worker force maintained through polymorphism should grant an advantage to adult colonies in much the same manner that pleometrosis does for starting colonies.

The natural history traits of V. pergandei discussed above appear likely adaptations to its major center of radiation, the Mohave Desert, where resources, especially favorable nest sites associated with moisture availability, are unevenly distributed in space. This results in clumping of starting nests likely within the vicinity of the maternal colony which, by definition has successfully reproduced and is, hence, an indicator of clumped resources. Pogonomyrmex rugosus, on the other hand, is most abundant in the Sonoran Desert where resources are more evenly distributed. At the interface of the Mohave and Sonoran Deserts (determined mainly by a cline where summer rains become less predictable) both species "track" aspects of the physical environment characteristic of their respective centers of distribution. This suggests that any competitive interactions between the species are secondary and that polymorphism in V. pergandei, like pleometrosis, is a response to intraspecific competition.

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Comparative Feeding Strategies in Two Harvesting Ants

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Messor capitatus is entirely sympatric with M. structor (with larger geographical distribution) in S. Europe. The two species are difficult to observe together, though, in some situations, they live close each other and in comparable densities. All our observations have been carried out in one such biotope, an abandoned field of about 3000 m² area near Sirolo (Ancona, Italy). Both species appear to be present in not too dissimilar numbers in the field, to randomly search for food with comparable speed, and, hence, with similar a priori probabilities of food discovery. Simultaneous measurements of circadian activity gave very different results according to microhabitat conditions (i.e. canopy, available food, etc.). Both species appear to be essentially diurnal in the field, at least in mid summer, but more nocturnal under standard laboratory conditions (Buser et al., 1986). Average nest populations as estimated by mark-recapture are about 850 workers for structor and ca. 1000 for capitatus. Both species forage individually but recruit nestmates to particularly abundant food sources. Under natural conditions, we guess the diet of both species as composed virtually only by seeds. They are, however, attracted by a variety of human sweepings - practically absent from our study area - and, if offered, clearly prefer dead insects and honey to seeds. We claim that the typical adaptation of several Messor species to dry environments and seed harvesting, merely represents the consequence of their reduced competitive ability as compared to other species. Our main purpose was to understand how two morphologically and apparently ecologically so similar species can coexist in the same biotope contradicting the so called "Gause's theorem" and the strictly vicariant microdistribution they show in most of their range.

Though nest entrances of these species are often only centimeters distant each other, intraspecific or interspecific battles are equally rare. Direct confrontation can be experimentally induced in the field by offering rich baits at strategical points already visited by workers from alien nests. In similar cases a single nest monopolizes the food source in a short time, but casualties are rare and most confrontation lies on a ritualized threatening display only roughly comparable to the one described by Hölldobler (1976) for Myrmecocystus. The display is obviously different in Messor, using different defence mechanisms, and involves mainly jerking with open mandibles and threatening with occasional snapping of the mandibles. Then, the colony successfully monopolizing a food source in such a way, builds a true barrier of "soldiers" between the food source and the competing nest. These "soldiers" are separately recruited from those visiting the bait and follow another trail from the nest. All previously described behavioural traits can be attributed to M. structor and/or capitatus according to the observation conditions. The food preference, tested on a sample of 12 seed species, also appears

to be virtually identical with a strong preference for wheat, the commonest culture in the area.

Artificially crushed seeds sieved and offered at different sizes also show virtually identical prey-size preferences for both species (ca. 3 mm diameter).

The main difference we have been able to observe between the two species lies on the trail system when they recruit to a food source: capitatus follows quite linear and narrow trails, while structor regularly uses much more irregular and quite broader paths (Table I).

Table I. Average number of ants ($\pm 2SD$) crossing an imaginary line through a trail from the nest to a 2 m distant food source during 2 min intervals. Counting line at 1 m from the nest. $n = 10$.

	Distance from the trail center in cm				
	50-30	30-10	+10	10-30	30-50
<u>M.capitatus</u>	.01 \pm .1	.2 \pm .2	76.7 \pm 32.4	.5 \pm .3	.2 \pm .2
<u>M.structor</u>	1.5 \pm .5	7.1 \pm 2.1	13.4 \pm 4.1	2.6 \pm 1.1	.2 \pm .2

Because this is practically the main difference we observed in the behaviour of the two species, we thought it might represent the key to understand their close coexistence. A first hypothesis is that capitatus, by performing a better recruitment, could secure more food from clumped food sources, while structor, by a greater energy investment, will cover larger areas and increase its chances to discover additional scattered sources. However, this is unlikely to represent a major factor in foraging efficiency because of: a) Messor trails in the study area are seldom very long and the discovery of new important food sources is progressively reduced by time; b) both species show additional, different reinforcement mechanisms only partly already known and understood in the literature. The best known reinforcement mechanism is the one shown by capitatus: this ant builds intermediate food storage points in the form of sets of piled seeds along the trail and there are ants carrying seeds only from the source to the storage point or from the latter to the next storage point or to the nest. M.structor has never been observed to build intermediate storages, but several individuals "leave" their seeds to a nest-mate or simply "lose" seeds under way. These abandoned seeds over a broader area are however collected by other ants carrying them to the nest. It appears hence that both species artificially produce the ideal food distribution better fitting their specific recruitment behaviour. These different foraging mechanisms and efficiencies, with structor showing better adaptation to rare and small food particles than capitatus and vice versa, are regarded here as sufficient to explain the close coexistence of the two species in the same biotope and as a further example of behavioural niche shift.

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Individual and Social Strategies of Foraging in the Ant *Formica schaufussi*

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Many ant species use both solitary and cooperative foraging. I am interested in how the resource and competitive environment of a colony affect decision-making processes of individual workers that influence prey selection and search behavior. In this paper I briefly describe the results of ongoing research with the ant *Formica schaufussi*.

Cost and benefits of solitary and cooperative foraging

The size of prey (dead insects) in the diet of *F. schaufussi* ranges from <1 to 220 mg, and 55% of the biomass of prey is cooperatively retrieved. The number of workers required to retrieve prey varies as a function of prey size, and prey transport time varies with prey size. Transport time was significantly correlated with prey weight, and the equation $\text{transport time} = .7265 - .0022 (\text{prey weight})$ was then used to estimate transport rates of prey. Net profitabilities (E/T) were then calculated on a per worker basis from the equation $E/T = \text{prey weight} / \text{transport time} / \text{mean number of workers retrieving prey}$. Results show that profitability is maximal for 32 - 64 mg prey; the shape of the profitability curve approximates that of the frequency distribution of prey in the diet. *F. schaufussi* workers seem not to forage on prey greater than c. 220 mg in weight because large prey require cooperative retrieval which reduces the per worker energy gain and they are unable to take large prey because they are lost to other ant species through interference competition. Therefore, workers should select individually retrievable prey rather than induce cooperative foraging to acquire larger prey. When given such a choice, foragers selected the smaller prey. In $N = 83$ binary choice tests the smaller prey were accepted in 76 trials (91.5%) and rejected 7 times (8.5%). Furthermore, of the 7 rejections, recruitment behaviors (trail-laying while homing) were observed 4 times (57%), indicating that in fact the forager had chosen the larger prey. *F. schaufussi* thus may be limited in the upper size limit of prey in its diet by the energetic costs of cooperative retrieval and the increased probability of loss to competitors.

The ecology and modification of search behavior

Foragers differ in their tendency to return to and continue to search at a location where food was found. Based on differences in area-restricted search (ARS) effort in the vicinity of the prior food find, persistent and non-persistent foragers could be recognized (Schmid-Hempel 1984). Persistent foragers show a high degree of ARS whereas non-persistent foragers soon leave the area, maintaining an angle approximately equal to that of their initial departure from the nest. Forager search patterns appear to be resource-correlated. Aphids and dead insects differ considerably in their spatial and temporal predictability, and seem to influence search effort. Components

of search behavior stimulated by either sucrose or insect prey were quantified to assess the spatial accuracy of search and its duration. Results show that persistent and non-persistent foraging patterns occur in response to both foods. Persistent foragers that had fed on sucrose showed a greater ARS effort than those fed insect prey, and comparisons of other components of search showed resource-related differences (Traniello 1986). Local search may also be modified by repeated contact with food of a given type, and more individuals shift from non-persistent to persistent search behavior after three consecutive rewards of sucrose. Also, search accuracy increases through repeated reinforcement with sucrose or insect prey. Reinforcement with insect prey, in contrast to either 0.5 or 1 M sucrose, produces a highly variable, less spatially accurate change characterized by greater ranging and a tendency to leave the reward site. Also, time spent in ARS is highly variable in response to rewards of insect prey and workers show little or no increase. A reinforcement schedule of 0.25, 0.5, or 1 M sucrose, however, produces a more consistent, higher level of search.

Discussion

Cooperative and solitary foraging permit a colony to track changes in resource availability. The costs and benefits of feeding on prey of different sizes appear to have influenced worker decisions to induce social foraging. Adjustments in forager search behavior may also permit individual workers to efficiently exploit foods with different distributions. Although colonies seem to produce persistent and non-persistent forager types in equal frequency, additional studies are needed on the development and modification of search behavior before any conclusions can be drawn. Furthermore, it must be determined if search behavior is constant throughout a forager's life and reflects specialization, and if it is age- or size-related. It will also be important to determine if the modification of search represents only a local change in behavior or if such change is more durable and indicative of learning.

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How Efficient Collective Behaviour Emerges in Societies of Unspecialized Foragers: the Example of *Tetramorium caespitum*

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Field data and laboratory experiments indicate that the foragers of *T. caespitum* are little specialized in their behaviour although they exploit a large diversity of resources either individually or collectively. We suggest that the colony's foraging activity is regulated by self-organization processes resulting from the interplay between stochastic events and amplificatory mechanisms. Self-organization is illustrated by the way the colony is able to select the most rewarding resources after recruitment. A mathematical model is able to simulate this flexibility, taking into account stochastic communication and non-linear trail reinforcement.

FIELD DATA

Foraging behaviour of a colony was observed on a pavement. The foraging field (about 12 m²) was exploited from various holes scattered over the foraging surface. The number and locations of the holes varied with time. Each exit hole corresponded to a definite sector in which ants foraged individually.

The distances from these holes at which the ants started to explore rarely exceeded 1 m, and their mode was shorter than 50 cm. Marking experiments showed that short-term memory to sites of food discovery and short-term sectorial fidelity were present, but the distribution of marked ants became more homogeneous only 24 h after marking. Individual foraging varied with season according to the availability of food. Simple feed-back mechanisms could regulate the abundance and distribution of the foragers if food density and distribution influence not only short-term memory of food location, but also the probability of foraging. This mechanism deserves more experimentation. The distribution of foragers resulted also from recruitments. Distant patches of seeds were exploited by long-lasting trails. New exit holes were progressively dug on these trails and the patches were incorporated into the foraging field as new sectors exploited from the new holes by individually acting foragers.

RECRUITMENT FLEXIBILITY

Recruitments in the field were observed to a wide variety of food sources. The mechanisms allowing the ants to allocate their efforts according to food quality were investigated in the laboratory. The experimental set-up corresponded to one sector of a natural foraging territory, explored from a single exit hole. Time-lapse photography and video-recording were used to follow the recruitment dynamics and to quantify the distribution of the ants in the foraging arena. Food sources were sucrose solutions of various concentrations located at 30 or 45 cm from the exit hole.

Recruitment to a single source (1M) appeared suboptimal. Even with-

out food exhaustion or overcrowding at the food source, a large proportion of the recruits explored the foraging arena without collecting. These ants are not considered as specialized explorers, but as lost recruits which missed the target. Experiments using artificial trails demonstrated that losing a trail is a stochastic phenomenon independent of the length of trail followed for a given concentration in trail pheromone. Trail-following behaviour improves non-linearly with trail pheromone concentration.

The value of "lost ants" appeared when 2 sources of unequal qualities were given in succession. Even when a recruitment towards a first source had reached its steady state, a richer source given later on was quickly discovered and more exploited. The distribution of the ants between the 2 sources depended on the difference of concentration between the 2 sources: the proportion of ants feeding at the poorer source was lower for greater differences in food qualities. Surprisingly, two symmetrical sources of equal quality were exploited unevenly, when given in succession or simultaneously.

SELF-ORGANIZATION DURING RECRUITMENT TO FOOD SOURCES

A mathematical model describing the recruitment to two identical sources was constructed on the following principles. Recruitment is described by the logistic-type equation, but only a fraction of the recruits reaches the source. Lost ants have a probability of finding one or the other sources or of returning to the nest. The fraction of ants reaching a source increases non-linearly as the trail is reinforced by the successful recruits.

The model and its properties will be fully described elsewhere (Deneubourg, Goss and Pasteels, submitted). The following equations describe the solution of a recruitment to two identical sources.

$$\dot{X}_1 = aX_1f_1 (N-X-E) - bX_1 + cE$$

$$\dot{X}_2 = aX_2f_2 (N-X-E) - bX_2 + cE$$

$$\dot{E} = a(X_1(1-f_1) + X_2(1-f_2)) (N-X-E) - pE - 2cE$$

$$f_i = X_i / (g + X_i)$$

Where X_i is the number of ants at one or the other source; $X = X_1 + X_2$; f_i the fraction of successful recruits; E , the number of lost ants; a , the recruitment rate; b , the departure rate; c , the probability of discovering a source by chance; p , the probability of the lost ants returning to the nest; g , a constant.

The analysis of the model's steady states indicates that when the number of participants is sufficient, the symmetrical exploitation of 2 identical sources is unstable as observed in the experiments. It further demonstrates that the stable asymmetrical exploitation is more efficient (as judged by the total number of ants feeding) than would be the unstable symmetrical exploitation. Thus collective foraging spontaneously evolves towards the most efficient pattern. What is true for this borderline situation is a fortiori true for more realistic situations when the sources are of unequal values and when trail reinforcement varies according to food quality.

Self-organization processes are probably widespread in insect societies and are an alternative or a complement to division of labour.

Foraging Strategy in *Formica bruni* in Relation to Colony Structure: an Important Step towards Polycalism

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Numerous biological and ecological studies have been made on evolved *Formica* species like the red wood ants. Nevertheless little is known about other less evolved species like *F. bruni* Kutter, belonging to the european subgenus *Coptoformica* Müller. Restricted to Switzerland, this species is known actually from five localities. We studied one colony located close to the Lake of Geneva shore about 20 km north-east of the city of Geneva (Feller and Cherix, 1984).

MATERIALS AND METHODS

Mounds nests of *F. bruni* were excavated carefully and all inhabitants collected to determine number of workers and queens. Internest exchanges as well as delimitation of territories were studied by hand marking with TechPen Ink colors. Concerning this last aspect, foragers were checked by direct visual observations three times a day (morning, noon, afternoon). Concerning foragers activity, we cut a strip of grass all around the nests and count the number of ingoing and outgoing foragers according to the main directions (north, east, south and west) during two 5 minutes periods each hour. For studying recruitment behaviour, we used baits (honey diluted with water) disposed on two concentric circles around the nest (respectively 1 and 2 m diameters), the first one with 8 baits, the second one with 16.

RESULTS

The studied colony involved at one time 61 active nests (1978) over an open area of 0.4 hectare. In 1983, only 18 nests are temporarily or permanently occupied by 7 societies (Feller and Cherix, 1985).

Excavation of one nest shows underground galleries leading to little chambers (about 1 cm) where the queens are located. This medium sized society contained 1'421 workers and 24 queens.

Foragers activity is strictly diurnal and of unimodal type in May-June and September-October (beginning and end of the active period) and of bimodal type in July and August, that corresponds to temperature over 40°C on nest or ground surface.

In spring, foragers are overdispersed on the entire area and no territory of one specific society is detectable. But later in summer, when foragers are mainly depending on honeydew produced by aphids on grasses (6 different aphid species) each society has its own territory. We found no aggressivity between foragers, moreover foragers of different nests did exploit the same territory even the same aphid colony.

The baiting experiment showed that the first forager discovering one food source will feed itself and return directly to the nest,

dragging its abdomen on the ground. Other foragers close to the trail showed immediately a high excitement and followed the trail directly to the source. The forager which laid the trail may stay some minutes into the nest before coming back to the source already occupied by other foragers. Fidelity to exploited baits is very high (over 70 %) and may last for several days. This is, according to Hölldobler (1976), a typical mass recruitment, where the trail has two functions : recruitment and orientation to the food source.

The studied colony presented the following kinds of social organization: societies inhabiting one single nest, societies inhabiting successively different nests during the active period and societies close together with workers exchanges (sometimes fusion between two or more societies).

DISCUSSION

Foraging strategy of F. bruni belongs mainly to the opportunistic type, that was confirmed by the different food sources exploited during the active period as well as the ability to change very rapidly from one source to another one, when for example honeydew producers disappear. This is possible by using trail pheromone. But what is more important is the ability to share trophic territory among foragers belonging to different nests without workers, brood and food exchanges. Moreover, moving from one nest to another one during the active period, fusioning with other societies are relevant factors which may lead towards true polycalism when food resources are stable or predictable and energy input in nest construction is very high like in the red wood ants. An intermediate case between F. bruni and Formica rufa group species is represented by F. truncorum (Rosengren et al., 1985) where internests movements in summer are important and support the hypothesis that the population distribution by this means could be adjusted to temporal and spatial variations in the resource distribution. In this case the authors described this kind of polydomous colonies as a supranest, an intermediate position between the case of F. bruni and some super-colonies of red wood ants (Cherix, 1980).

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Mixed Feeding Strategies in Ants

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When wood-ant workers forage on trees there seem to be no distinct classes of hunters and collectors of honey-dew: a worker returning to the nest with prey on one occasion may have honey-dew in its crop on another. Each forager moves through the tree, probably towards or around a group of aphids whose location is familiar to it. During these movements it may encounter an aphid and accept a drop of honey-dew, or a prey which it may capture. It may also, at least after mid-summer, attack and capture an aphid. Which of these opportunities it has will depend on the relative abundance of aphids and prey; which option it actually takes will depend on its tendency to accept or refuse honey-dew, and its tendency to attack or avoid prey.

We have shown (Sudd & Sudd, 1985) that the probability of acceptance of a sugar solution by an ant on a tree, depends on its concentration, and can be modelled, as might be expected, by a probit regression. This model assumes that each ant has, at the time of the experiment, a threshold sucrose concentration. It accepts sucrose of this or a higher concentration, and refuses lower concentrations. In the population of foragers thresholds vary in a Gaussian manner about a mean threshold. This mean can be estimated for the population as the Median Effective Concentration or ED50. We were able to show that there were marked seasonal changes in this value.

1. The ED50 was low in spring and rose very sharply over a period of less than a week in late June. The variance in the population, (modelled as the slope of the probit regression), remained constant.
2. The change was almost simultaneous in ants on pine and on birch trees, but the responses of ants on their way to trees were not quite the same.
3. The model was a satisfactory fit, provided that these seasonal differences were incorporated in it.
4. The increased threshold sugar concentration among collecting workers in June explained the desertion of the extra-floral nectaries of bracken *Pteridium aquilinum* at precisely this time.
5. The time of the change also coincided with the maturity of the first (fundatrigeniae) generation of *Cinara pini* on pine and *Symydobius oblongus* on birch; these were the aphids most attended.
6. The time of the change coincided with the reduction in population numbers or entry into summer diapause of other aphid species e.g. *C. pinea*, *C. pilicornis*, *Thelaxis dryophila*.
7. Over the whole season no wood-ant forager ever accepted pure water.

We concluded that the change in ED50 represented a change in the demand for sugars in foragers, and presumably in the colony. In a laboratory nest we were able to show that the ED50, measured in the same way, of ants leaving the nest was closely related to the concentration they had been offered over the previous few days. Ants pre-

treated for 3 days with distilled water alone had an ED50 of less than 0.1M sucrose; when sucrose as well as water was supplied during the during the pre-treatment foragers had ED50s a little lower than the concentration supplied as a pretreatment (Sudd & Sudd, 1985).

Since July is a period when wood-ants attack aphids, which may form a large proportion of the prey taken to the nest at that season, we next considered whether changes in responsiveness to sugar might affect the treatment of aphids by the ants that tend them. We did not investigate responses to aphids directly, but instead offered ants tending aphids a dead *Drosophila*, impaled on a hypodermic needle. We were then able to offer the ant a standard prey, with a drop of sucrose solution of known concentration, or without such a drop. In general we found responses to sugar alone reflected those we found in 1982, 1983 and 1984. We also found that ants would always accept a fly without sugar. Presentation of fly and sugar together models the encounter of an ant with an aphid, which could be taken as prey but offers honey-dew. The interesting results of this experiment are shown in Table 3 and figure 1.

TABLE 3
THE RESPONSES OF WOOD-ANTS TO A FLY AND SUCROSE PRESENTED TOGETHER

MODEL	DEVIANCE	DF	DEV.CHANGE	DF CHANGE	SIGN.
GENERAL MEAN	66.15	30	-	-	-
SEASON MEANS	60.65	29*	5.50	1	*
(a) SPRING	24.07	13	-	-	
LOG-CONC	12.67	12NS	11.41	1	*
(b) SUMMER	36.59	16	-	-	
LOG-CONC	35.86	15*	0.72	1	NS
SEAS. LOG-CONC	48.52	27	-	-	
DAY, SEASONS	9.71	21NS	38.81	6	*
DAY, LOG-CONC	5.79	15NS	3.92	6	NS

NS = NOT SIGNIFICANT AT $P = .05$; * = significant at $P = .05$ or less.

In spring, when demand for sugar is high, sugar is able to compete with prey, and higher concentrations can prevent attack. In summer however there was no level of sucrose concentration which protected a fly from attack, although the mean rate of attack varied considerably from day to day. While this fits the idea that some aphids and extra-floral nectars may not attract ants in summer, it also means that no aphid is likely to be protected from predation by virtue merely of the sugar content of its honey-dew. We must look either to other components of the honey-dew, or to behavioural or chemical defences of the aphid.

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Size of Colony and Foraging Territory of the Desert Subterranean Termite, *Heterotermes aureus* (Snyder): a Preliminary Report

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Heterotermes aureus (Snyder), the desert subterranean termite, is the most common of the subterranean species in the Sonoran Desert below ca. 1,220 m. Its population density has been estimated at 4.31×10^6 individuals/ha in a desert grassland on the Santa Rita Experimental Range 40 km south of Tucson, Arizona (Haverty *et al.*, 1975). An estimated 190.4 colonies occur per ha, with an average foraging territory of 12.5 m². However, the latter estimates were qualified as perhaps being somewhat arbitrary, and Haverty *et al.* (1975) suggested that the groupings might also represent subcolonies or foraging areas of much larger colonies. Thus, we initiated further research to examine the foraging ecology of *H. aureus*, using similar but somewhat more sophisticated techniques.

MATERIALS AND METHODS

Study Site.--Studies were conducted on the Santa Rita Experimental Range at an undisturbed site adjacent to that used by Haverty *et al.* (1975).

Territory Size.--Foraging activity of *H. aureus* was studied in 30 plots, each consisting of a 6- by 6-m grid of 25 toilet paper rolls spaced at 1.5-m intervals. Plots were selected on the basis of two criteria: (1) the presence of *H. aureus* foragers in dead wood, and (2) separation by at least 12 m from any other plot. Most plots were separated by not more than 50 m from at least one other plot.

A dye, Sudan Red 7B, was used to mark termites and identify the territorial extent of colonies. The dye is retained in the insect fat body for several weeks (Su *et al.*, 1983), so dyed termites were released on a plot-by-plot basis with approximately a 1-month interval between subsequent releases of dyed termites in any adjacent plot.

Colony Size.--The numbers of *H. aureus* foragers per colony were estimated using a Lincoln index, mark recapture technique. This was done concurrently with the determination of colony extent, except that toilet paper rolls containing termites were returned to the laboratory and actual counts of white and pink termites made.

In another series of experiments, an exhaustive trapping technique was used to estimate numbers of foragers per colony. Initially, field locations were identified that contained *H. aureus* foragers in dead standing or fallen cholla cactus (*Opuntia* spp.). The infested wood was removed, then five rolls of dampened corrugated fiberboard were fitted

into polyvinyl chloride (PVC) sleeves (10 cm by 15 cm) and buried at each site (LaFage et al., 1983). Six sites were established in July and August 1984, and another two in April 1985. Since then traps have been examined and replaced approximately once a month. We assume that each of the eight sites continues to be a sampling of the original colony. Termites from each of these sites have been destructively sampled and counted. The counts include those found in the wood that was initially removed from each site, as well as termites from the fiberboard traps.

RESULTS AND DISCUSSION

Territory Size.—Although marking studies are incomplete, initial data suggest that the foraging territory of an H. aureus colony is probably larger than was estimated by Haverty et al. (1975). In five situations, termites belonging to the same colony were found foraging in two or more distinct plots. In one case, the plots were separated by 34 m. The largest territory exceeded 200 m².

Colony Size.—Based on mark recapture data from five foraging territories, estimated numbers of foragers range from $67,819 \pm 1,463$ ($\bar{X} \pm SD$) to $302,411 \pm 1,331$ termites. These figures are much higher than the previous estimate of 22,632 termites in an average H. aureus colony (Haverty et al., 1975).

Numbers of termites collected from the eight sites with fiberboard traps during the first year after establishment are as follows: col. 1 - 7,210; col. 2 - 2,604; col. 3 - 5,667; col. 4 - 1,726; col. 5 - 4,130; col. 6 - 41,420; col. 7 - 75,166; and col. 8 - 35,070. These sites are still being monitored and the trapped termites removed. During the second year of study (up to May 1985), numbers of termites collected had approximately doubled for colonies 2, 4, and 6; approximately tripled for colony 1; approximately quadrupled for colonies 3 and 5; and more than 97,000 termites had been removed from colony 7. Obviously, removing termites over an extended period of time will allow them to rebuild colony numbers to some extent. However, the intrinsic rate of population increase for termites is low, and it is unlikely that the colonies can replace the large numbers of individuals that they continue to lose month after month.

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Foraging Strategies of Grass-Cutting Ants, and the Use of Environmental Grain in Comparison with other *Atta*

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Various studies have attempted to explain the foraging patterns of leaf-cutting ants of the genus *Atta*. Most of these studies have been performed in complex tropical rain forests, where a high species diversity and a complex three-dimensional structure do not permit the simultaneous testing of alternate hypotheses of foraging patterning (Fowler and Stiles, 1980). By studying the foraging patterns of grass-cutting ants, further resolution of the spatial patterning of resource exploitation can be obtained, due to a two-dimensional habitat, with species diversity being controlled for by using planted (*Pangola*), and natural mixed species pastures. These results are then fitted to a simple optimization model of spatial use, and compared with patterns reported for tropical forest leaf-cutting ants.

MATERIAL AND METHODS

In planted and natural pastures, respectively, 5 colonies of the grass-cutting ants, *Acromyrmex landolti*, *Atta bisphaerica*, and *Atta capiguara* were marked for study in the vicinity of Rio Claro, state of Sao Paulo, Brazil. The foraging trails of each of the 10 *A. capiguara* and the 10 *A. bisphaerica* nests were mapped monthly, and pitfall traps located at 1 m intervals along these trails and extending into the habitat were emptied for three 24 hr monthly periods. For the *A. landolti* nests, the distances and directions of marked workers from each nest were recorded for 10 monthly intervals. For *Atta* nests, concentric 5 m rings were superimposed upon colony maps, and recording foraging and cutting in each ring during 10 monthly observations. The spatial usage of trail development, trail growth, and for harvesting activity were tested for heterogeneity. These data, along with the captures of ants in pitfall traps, were incorporated into a simple model of spatial usage by each species of *Atta*.

RESULTS

Colonies of *A. landolti* never foraged in a 360° pattern. Only one section of the habitat was used, forming linear territories for foraging. No significant differences were found in spatial dispersion nor maximum distance foraged for colonies in planted and natural pastures. However, in planted pastures, foraging activity was found to be more concentrated nearer the nest than in natural pastures. Colonies of *A. bisphaerica* and *A. capiguara* formed fewer,

longer trails in planted pasture, and more, but shorter trails in natural pasture. Monthly rates of change in trail dispersion, trail length, and trail numbers were, however, not significantly different between natural and planted pastures. The patchiness of cutting activity of these species of Atta, were found to be significantly different between species, but not significantly different within a species, irrespective of pasture type. For both species of Atta, foraging ants trapped with pitfall traps declined significantly with distance from the foraging trail, and combined data for both species monthly studies fit a Poisson distribution.

DISCUSSION

These results, although from species living in simple two-dimensional habitats, are similar to those reported for Atta in complex three-dimensional habitats (Fowler and Stiles, 1980). Because of the Poisson distribution of foragers found with respect to distance from a foraging trail, as well as a higher probability for an existing trail to grow rather than a new trail to be formed, trail development can be modeled by autocatalytic growth equations. Trails are thus Markovian products resulting from an area restricted search (Fowler and Stiles, 1980). Trails result in a higher energy flow (Fowler, 1982), resulting in a greater variance of energy, and a greater structural stability. A model of trail use and development using partial derivatives which shunt stored energy into trail lengthening and new trail formation, due to the higher variance, results in trail lengthening. Energy return per unit time is greater than without trails (data simulated), and is greater nearer the existing trails, as found through spectral analysis. The patterns reported for complex three-dimensional forest species of Atta, may be nothing more than simple consequences of the energetics of trail use and formation, exploiting the spatial variance of vegetation suitable for fungal substrate. The influence of plant chemistry may be only white noise in the spatial exploitation of a habitat by these ants.

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Foraging Strategies of Seed-Harvesting Ants in the Pinacate Desert, Mexico

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Many species of seed-harvesting ants of the genus *Pogonomyrmex*, *Veromessor*, *Pheidole* and *Novomessor* are common insects in the semiarid regions of Mexico. We have registered in the Pinacate desert area 20 species of ants, which the seed harvester group comprises 10 species.

Among the desert seed-eating ants, differences in colony foraging behaviors are associated with resource subdivision on the basis of seed density and spatial distribution on the ground and appear to promote coexistence between species with similar food requirements (Davidson, 1977).

Seed resources in deserts are not continually renewable, but occurs through short and periodical pulses of production followed by long periods of reserve with a gradual decline in seed density due to removal by ants, rodents and birds and climatic factors as winds. The basic strategy of the seed-eating ants is to optimize foraging behavior when these periodic pulses of production occur and to harvest very large stores of seed under the ground which can function as a reserve during the periods of resource scarcity.

The purpose of this investigation is to determine how foraging strategies may be related to seed resources production in communities of desert seed-eating ants.

MATERIAL AND METHODS

The Pinacate desert is located in the Northwestern coastal plain of the gulf of California, Sonora state, comprising the Sonoita river and the Altar desert. The mean annual rainfall is approximately 60.7mm. and the natural vegetation is composed by a microphyllous desert with *Larrea tridentata*, *Ambrosia dumosa* and *A. deltoidea* as characteristic species. The ground between these shrubs is occupied by a diverse flora of annual and perennial grasses and forbs.

We have been working with three species of seed-eating ants: *Pogonomyrmex* sp., *Veromessor pergandei* and *Novomessor cockerelli*, in three selected sites. Two quadrats (50x50m) were set up in each study site. Estimates of colony numbers were obtained by counting nest entrances. The composition of the diet has been determined by removing seeds from returning workers. Collections and data of the nest debris were also obtained. Estimates of plant density and seed production were made using random quadrats (1m²). Field work was conducted at two intervals which coincide with periods of scarcity and overabundance of seed resources, from late winter 1981 to spring 1986.

RESULTS

Foraging methods observed in seed-eating ants, can be broadly categorized as: individual foraging, group foraging and recruit foraging (Bernstein, 1975; Davidson, 1977). Foraging behavior in desert ants, depends on a number of factors of the food source, such as distance to the nest, density of the seed fall, size and quality of the grains and the presence of other species. Based on manipulations with Veromessor pergandei, Bernstein, 1975, shows that group foraging is adaptive for feeding on low density seed resources. She indicates that workers forage individually during the period of seed production. We did not find significant changes in the foraging strategy of V. pergandei between periods of high and low resource abundance. Foragers may travel in well defined columns, over distances as great as 20 meters in search of food, during the season of high food resources.

During this period, V. pergandei collected predominantly the massive seeds of the forb Dalea neo-mexicana and the grass Schismus arabicus. In contrast, the individually foraging Novomessor cockerelli and Pogonomyrmex sp. collected seeds of diverse taxa (eight and six species, respectively) more equitably and occasionally some insects.

These data can suggest that within certain limits the harvester ants will concentrate on freshly fallen seeds, specially those which are overabundant and occur in patches. V. pergandei usually started to forage in the morning, when the surface temperature was 15-20°C and continued until the surface temperature reached 30-35°C. During the summer the rapid increase of the surface temperature (20°C from 10.00 to 14.00) is the major factor for terminating the foraging activity in these ants. Species of the genus Pogonomyrmex, gathered from at least eleven and up to thirty-nine kinds of seed (Willard & Cromwell, 1965). Tevis, 1958, listed seeds of fourteen different species in the diet of V. pergandei, we found that during the period of low seed density, V. pergandei collected at least thirteen seed species. Preliminary field data in the Pinacate desert, showed that there was not a direct correlation between the proportion of seeds in the diet of V. pergandei and their relative abundance on the ground.

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Foraging Strategies of Fire Ants

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The species in neotropical Solenopsis saevissima and S. geminata complexes are commonly known as fire ants, due to their fiery, itchy stings. The mono- and polygynous forms of these conceptually multi-dimensional species are generally sympatric. Their dispersion may be characteristic of their foraging-nesting strategies.

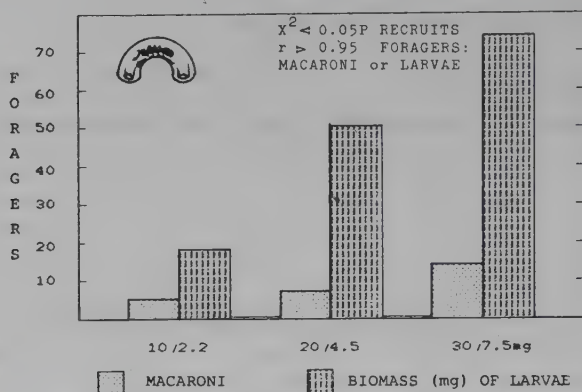
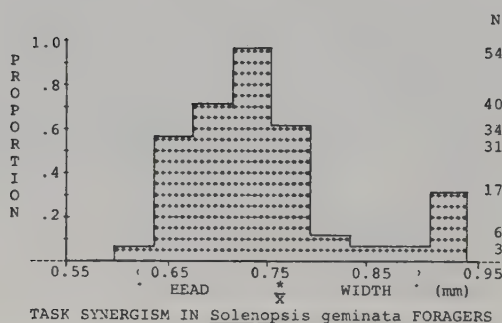
MATERIALS AND METHODS

The general deductions on foraging are drawn from the investigations on S. geminata in Tabasco, Mexico (1980-84), and S. invicta in Florida (1969-75) and Texas (1984-86). The forager recruitment to Vigna and Phaseolus nectaries, stored products pests (Sitophilus zeamais, Tribolium confusum, bruchids) in the field (Bhatkar 1982) and human dwellings, and to 100 mg of tuna fish baits was investigated. Foraging by outdoor S. geminata colonies to different biomass of T. confusum larvae in household macaroni (1 larva/macaroni) was observed. The fire ants are known to forage in human dwellings during extreme dry and wet periods.

RESULTS

The fire ant foraging involves, 1) limited epigeic fan searching by foragers dispersing from the hypogeic tunnels, 2) chemical mass-recruitment of nestmates, 3) concealed (hypogeic) transport of food, 4) covering a constant resource (nectaries, animal tissue, etc.) with soil encrustation against the competitors, 5) proliferation of hypogeic foraging tunnels to the resource, 6) transport of older larvae to protein-lipid rich food source, and 7) building storage mounds to extend the colony up to the resource. The monodomous, monogynous forms monopolize food stations and the colonies fluctuate temporally and spatially during resource exploitation. The polydomous, polygynous colonies seem to forage uniformly and tenaciously even through 30-36°C temp. The forager recruitment appears to be directly correlated to the amount or richness of food in protein or lipid. The size of the load seems to match the size of the forager. In these truly polymorphic species, where the worker size [5 times max. head width (hw)] otherwise approximates a continuum of 0.5-1.5 mm hw in invicta and 0.5-2.5 mm hw in geminata, the forager recruitment tends to be bimodal (Fig). Thus, a large group of smaller media (0.6-0.8 mm hw) and a small group of larger media (0.8+ mm hw) become "task

Fig.

RECRUITMENT RELATION OF *S. geminata* TO MACARONI & AND INFESTING *Tribolium* LARVAE

synergists." The extreme sizes are rarely recruited as foragers. The major workers seem to specialize in seed crushing, storing of liquid food or defending the colony either passively or actively. Intercolonial trophallaxis at the foraging boundaries occurs as a means to avoid contests amongst the competing forms (Bhatkar 1979).

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A Simple Model to Simulate the Organisation of Individual Foraging in *Neoponera apicalis*

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Neoponera apicalis is characterised by a small colony size (± 100 adults). It is an individual and diurnal forager, the prey being small insects and larvae. It is possible to distinguish two types of foragers :

- 1°) Close to the nest, the population density is high, and the animals forage on overlapping individual zones.
- 2°) Far from the nest, a marked spatial fidelity may be noted. These foragers, have a "personal" zone to which they return more or less regularly over a long period of time (1 month and more). These ants are more frequently outside and capture more prey than the unspecialised foragers hunting close to the nest.

HOW COULD THE FORAGERS ACHIEVE THIS ORGANISATION?

From experimental data, it appears that this foraging pattern is the result of a learning process, whereby an ant that returns to the nest with a prey stays less time in the nest and returns to the zone where it captured the prey. This hypothesis has not yet received clear experimental demonstration. However, a minimal mathematical model shall show that, in the total absence of any communication between the foragers, learning is able to generate :

- an efficient distribution of the foragers in the foraging area as a function of different characteristics such as the degree of inhomogeneity.
- a repartition between foragers and non-foragers.
- a foraging pattern close to that of *Neoponera apicalis*.

We deliberately ignore all other factors, such as age, which could be involved in the foraging pattern's genesis.

SCRIPT OF THE MODEL

At the beginning of their life the ants are similar and characterised by a probability $Q(0)$ of foraging and have a probability $P_i(0)$ of going to foraging zone i . If it catches a prey, its probability at the next time-step of leaving the nest increases by q^+ and of returning to the zone of capture increases by p^+ (p^+ & q^+ are the learning rates). If the ant does not catch a prey, its probability of leaving the nest at the next time-step decreases by q^- (the rate of forgetting) and the difference between the probabilities of going to the different boxes decreases. Each zone is characterised by its food dynamics. In the present case, each zone has a probability r_i of containing a food particle at

each time-step (For a detailed description of the model, Deneubourg et al., in press). The system reproduces the spatial learning and/or the activity learning.

SELECTION OF THE BEST ZONE

In the case in which one zone is richer than the other, the best strategy is to develop rapidly a fidelity to it. The benefit (prey captured/step) as a function of the rate of learning exhibits a maximum value. The corresponding optimal rate of learning is a function of :

- the foragers' life-time : if the life-time is longer the optimum is reached for a lower rate of learning and the corresponding benefit is higher.
- the heterogeneity of the environment : if the heterogeneity is higher, the optimal rate of learning is higher as is the corresponding benefit.

OPTIMAL SPATIAL DISTRIBUTION IN A HOMOGENEOUS ENVIRONMENT

In a homogenous foraging area ($r_1 = \dots = r_n$) patrolled by k foragers of the same colony, the "optimal" solution is to have k/n specialized foragers in each box. The model is able to drive the society spontaneously towards this optimal configuration with a corresponding optimal rate of learning.

OPTIMAL SPATIAL AND TEMPORAL DISTRIBUTION

If the two learning processes (leave the nest & choose the zone) interact, we observe the spontaneous generation of two sub-populations :

- 1) most leave the nest occasionally, without spatial fidelity
 - 2) others leave the nest often, with spatial fidelity.
- These two sub-populations correspond \pm to the two Neoponera apicalis forager types : inactive, unspecialised near the nest & active, specialised far from the nest.

Varying the number of ants and the food density shows that the learning processes are able to adapt the number of foragers to different situations, thereby increasing the foraging efficiency and keeping a number of ants in reserve for others tasks.

DISCUSSION

This model is close to Wehner's script (in press) for Ocymyrmex or Cataglyphis (Wehner et al., 1983), which have a strategy similar to N. apicalis. It could also be used for task specialisation.

A lot of \pm complex learning models have been proposed. However only a small number see learning as a mechanism of social structuration.

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A Model of Individual Foraging: the Factors Influencing an Ant Society's Foraging Energy Budget

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THE MODEL

Uniform food sources arrive and disappear randomly at a fixed rate in the circular foraging area. A fixed number of foragers search the foraging area. When one encounters a food source it transports a load to the centrally-sited nest and returns to the foraging area. With memory of the food source location, the ant returns to the source and transports another load until the source is exhausted. Without this memory the forager searches directly for a new source, even if the first source is not yet depleted.

The model quantifies the interactions between the foragers and the sources by means of differential equations :

$$d\text{Sources}/dt = \text{Arrival} - \text{Capture by Foragers} - \text{Competition}$$

$$\text{Net Benefit} = \text{Joules Captured} - \text{Forager Consumption}$$

The model is used to evaluate the influence on the colony's energy budget of four parameters :

- i) the forager size, which influences the foragers' consumption, their load capacity, the rate at which a forager encounters a food source and the number of loads contained in one source.
- ii) the sources' availability time, i.e. the average time for which a source is available to the colony in the foraging area, being an inverse measure of the competition.
- iii) the size of the sources, which influences the number of loads per source and their arrival rate.
- iv) the foragers' memory of the food source location.

As a Joule is worth more to a large ant than to a small ant, in order to be able to compare different sized ants' benefits we define a standard benefit as being the net benefit divided by the average consumption of a non-foraging ant, thus indicating the number of larvae and domestic workers and reproductives that the foragers can feed.

RESULTS

Firstly, how does the standard benefit vary as a function of the number of foragers and of the foraging area? For a given number of foragers there is an optimal foraging area and for a given foraging area there is an optimal number of foragers. These two factors combine to give a unique maximum benefit, at which each forager is able to feed typically between one and two non-foraging ants. However it is

well-known that for every forager in an ant society there are in the order of 10 non-foragers, and thus this maximum benefit is incompatible with the ants' social organisation. If we therefore assume that each forager must feed at least 10 non-foragers, the maximum "compatible" benefit (called here the optimal benefit) is much less than the maximum possible benefit, as is the corresponding optimal number of foragers and foraging area. Larger ants have larger optimal foraging areas but lower optimal foraging densities.

How does this optimal benefit vary as a function of the food size, for different sized foragers? Without memory of the source location the maximum optimal benefit is obtained when the foragers exploit sources equal in size to their load capacity. This maximum is a sharp peak, meaning that if the foragers exploit sources smaller or larger than their load capacity, their benefit is greatly reduced. With memory of the source location this peak becomes a plateau, meaning that the foragers can exploit a wide range of food sizes with near-maximum optimal benefits. Memory thus reduces the constraint equating ant size and food size. The benefit with memory is always greater than or equal to the corresponding benefit without memory, depending on whether the source is greater or smaller than the foragers' load capacity. Finally, there is a best ant size, in that there is always one ant size whose maximum optimal benefit is higher than that of other sized ants. Increasing the sources' availability time (reducing competition) increases the optimal benefits for all ant sizes and increases the best ant size.

Let us compare the relative performances of a small ant with and without memory and a large ant with and without memory as a function of the sources' size and availability time. We note that for small sources the small ant achieves a higher optimal benefit than the large ant and for large sources the inverse is true, independently of the availability time. For intermediate sized sources, with long availability times the large ant with memory has a higher optimal benefit than the large ant without memory which has a higher benefit than the small ant with memory. As the availability time decreases (increasing competition) the small ant with memory moves up from third place to second place and finally to first place. Whether it is better to have the right size or to have memory thus depends on the sources' availability time.

Unlike in the model, a society's foraging area is limited by the foragers' capacity for returning to its nest and by the territoriality of neighbouring societies. This limit would affect larger ants more than smaller ants as we have seen that they have larger optimal foraging areas. If we now consider territoriality as a way of increasing the availability time of sources' in the defended foraging area, the above results would suggest the following two associations: large societies of large, aggressive, territorial foragers, exploiting large prey in large foraging areas with low foraging densities or small societies of small, non-territorial foragers, exploiting small prey in small foraging areas with high foraging densities.

The Spatial Distribution of Foraging Effort in *Myrmica rubra* (L.)

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INTRODUCTION

This study investigates the manner in which *Myrmica rubra* workers utilize the area around their nests. *M. rubra* is a generalist forager; its diet consists of food gathered by tending aphids, by scavenging (for carcasses and for liquids) and by active predation. In addition to the standard problems of optimisation (see e.g. Pyke 1984) ants are confronted with two further difficulties. As central place foragers, the ants must expend time and energy travelling between the nest and the area to be searched. Aronsen and Givnish (1983) suggest several mechanisms whereby the distribution of foraging effort may be maximised. The second difficulty arises because ants are social insects; foragers might be in competition with each other if the success of one reduces the food availability (and the chances of success) for another. How then might a colony of *Myrmica rubra* distribute its foraging effort over the available area?

METHODS

To investigate this, observations were made on the foragers of two nests from which a number of individuals were followed upon leaving the nest for the duration of their foraging run. Some 25% were seen to visit plants carrying aphid clusters; 15% were lost after several minutes; and 3% collected insect carcasses. The positions of the individuals were recorded every 30 seconds (shorter times were impractical). The trails so plotted were analysed to reveal the distance and bearing of the ant from the nest at each 30 second interval. Rayleigh tests (Batschelet 1981) were used to see if this sample of bearings was clumped or evenly distributed. A Friedman analysis of variance by ranks was used to see if any of the ants searched at different distances than others. For these analyses only the first nine minutes (at most) of any trail were used; this allowed incomplete trails to be included.

RESULTS

The first analysis revealed that the bearing of any individual ant at any time was significantly clumped around its mean

bearing for that foraging run. Moreover when all the ants were compared it was shown that they too were significantly clumped around the grand mean. For both nests this grand mean was also significantly similar to the bearings of the plants carrying the most-tended aphids (for one nest the grand mean was 079° , while the aphid-carrying plant, 2m away, was on a bearing of 086° ; for the other nest the mean bearing was 187° , the bearing of the plant with the aphids was 245° , at a distance of 1m). There was no significant difference between ants in their distances from the nest. That is no individual was to be found consistently closer to or further from the nest than any of the other foragers.

DISCUSSION

It is seen from this that some areas surrounding the nest are searched more frequently than others, as predicted by Carroll and Janzen (1973). The area can be described as an arc extending towards a permanent food supply, even though this source was not exploited by many of the foragers which travelled in this direction. There is no evidence that this arc was subdivided further by individuals restricting their searching to specific distances from the nest. How might such a pattern arise? It may be that the area with the tended aphids also contains the highest density of other foods. Alternatively the nutritional requirements of the colony may not be so great as to demand that all the potential area need be searched. Another explanation could be that only very few foragers are needed to scout the remaining areas effectively, too few to have affected the general clumping pattern, yet sufficient to exploit any food they might encounter. It is also possible that the division of labour which makes up the mixed foraging strategy may reduce competition between nestmates, thus eliminating the reason for the foragers to spread their search effort in the first place.

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Trail Polarity in the Ant *Monomorium destructor*

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Many species of ants lay recruitment trail substances which serve to draw their nest-mates to newly found food resources. Whether these substances are polarized such that ants obtain directional information from them has been a topic of contention. This idea, first proposed by Bethe(1898), has been supported (Macgregor,1948), refuted (Carthy, 1951; Wilson,1962) and reviewed (Wilson, 1971; Leuthold,1975). We performed field experiments on this phenomenon in *Monomorium destructor*, and our results suggested strongly the polarity of direction in the trail of this species.

MATERIAL AND METHODS

Monomorium destructor (Jerdon) is a pantropical species which probably originated from Africa. The northern limit of its distribution in East Asia seems to lie in Okinawa Island, Japan, where the present study was carried out. This species nested in the holes of living trees and in the crevices of exposed limestone and concrete buildings. The species was commonly observed to construct foraging parades and to carry dead animals such as insects and spiders.

Food (termites of *Coptotermes formosanus* and baby food) was provided in petri dishes set 1 m apart from the nest opening of *Monomorium destructor*, and two elongate wooden pieces were placed in a line between the food resource and nest opening. About one hour later, many workers went to and came back from the food resource, forming a foraging parade. One of these two wood pieces (food side piece) was then taken, the workers on it were removed with a forceps, and positioned so as to make a T-shape with the other piece. The direction taken by departing workers upon reaching the T-junction (ie. nest or food side) was noted for 5 or 10 minutes. The orientation of the wood piece was then reversed and the same observation made. Similar experimental manipulation were also performed on workers returning to the nest.

RESULTS

Table 1 shows the number of departing workers which turned to the food or nest side at the T-junction. About 75 % of the workers turned to the food side. Even when the orientation of the wood piece was reversed, the results were the same. However, workers returning to the nest from the food showed no clear turn-choice preference (Table 2), although slightly more workers seemed to turn to the nest side. These results cannot be explained without assuming a polarity in the trail of this species, although the cues used are, at present, unknown.

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Table 1. The number of departing workers which turned to the food side and nest side at the T-junction. The wooden pieces are 20 cm in length.

Number	Total number of workers	No. of workers which turned to nest side(%)	food side(%)	Number	Total number of workers	No. of workers which turned to nest side(%)	food side(%)
Standard				Reversal			
1	75	3(4)*	72(96)*	2	69	10(14.5)*	59(85.5)*
3	80	16(20)*	64(80)*	4**	43	5(11.7)*	38(88.3)*
5**	34	5(14.7)*	29(85.3)*	6**	38	6(11.2)*	32(88.8)*
7**	45	8(17.8)*	37(82.2)*	8**	40	9(22.5)*	31(77.5)*
11**	37	9(24.3)*	28(75.7)*	18**	38	11(29.0)*	27(71.0)*
14**	44	8(17.8)*	36(81.8)*	20**	47	14(29.8)*	33(70.2)*
15**	38	16(42.2)	22(57.8)	23**	51	17(33.3)*	34(66.7)*
16**	29	8(27.6)*	21(72.4)*	29	70	18(25.8)*	52(74.2)*
17**	42	11(26.2)*	31(73.8)*	33	69	17(24.7)*	52(75.3)*
19**	36	14(38.9)	22(61.1)	38	83	18(21.7)*	65(78.3)*
22**	49	13(26.6)*	36(73.4)*	40	68	14(20.6)*	54(79.4)*
28	75	19(23.4)*	56(74.6)*	44	75	22(29.4)*	53(70.6)*
32	71	19(26.8)*	52(73.2)*	47	67	15(22.4)*	52(77.6)*
34	67	20(29.9)*	47(70.1)*	50	64	14(21.9)*	50(78.1)*
37	89	17(19.1)*	72(80.9)*	67	55	12(21.8)*	43(78.2)*
39	65	13(20)*	52(80)*	70	83	22(26.8)*	61(73.4)*
43	91	31(34.1)*	60(65.9)*	84	80	23(28.8)*	57(71.2)*
45	76	18(23.6)*	58(76.3)*				
46	92	23(25)*	69(75)*	mean		(23.3)	(76.7)
48	66	18(27.3)*	48(72.7)*				
49	74	15(20.3)*	59(79.7)*				
58	63	12(19.1)*	51(80.9)*				
66	54	8(14.9)*	46(85.5)*				
69	146	34(23.3)*	112(76.7)*				
83	78	22(28.3)*	56(71.7)*				
mean		(23.9)	(76.1)				

Interactions of Orienting Mechanisms by the Red Wood Ant in a Three Dimensional Foraging System

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INTRODUCTION

Cosens and Toussaint (1985) recently showed that red wood ant workers belonging to the *Formica rufa* group can be trained to feed at a food source located at a fixed spatial position in a three dimensional foraging system. This observed site allegiance and route fidelity rises the question of the orientating mechanisms involved in such a multidimensional foraging system. The aim of this study is thus to investigate these mechanisms and see how they interact with and substitute each other in various experimental situations.

MATERIALS AND METHODS

In order to disentangle the intricacy of these mechanisms tests were carried out in controlled laboratory conditions. A specially designed experimental set-up was used for the tests. A vertical downpipe topped by a horizontal cross shaped bar is driven directly in the colony mound. The two parts of the system can be rotated independently around the vertical axis. The system is lighted from the left side by a directional light.

Each experiment was preceded by 2-3 days of starvation. Ants were food rewarded with raw cane sugar at the extremity of one of the branches about 1h before each test. It must be pointed out that the food remains in this same spatial position throughout the whole test and whatever the experimental situation. Each test lasted half an hour. As soon as they climb over a line, 30cm below the cross, ants are individually followed till they reach the extremity of one of the bars or otherwise turn around to go back to the nest.

RESULTS

1°) Vertical axis

The first control experiment corresponds to the standard situation prevailing during the hour preceeding the beginning of the test. Ants unanimously ascend the lighted side of the pipe.

In the second control situation, the directional light is switched off and the experimental set-up uniformly lighted from the ceiling. Furthermore, any potential chemical cues are removed by washing the pipe off with diluted alcohol. In this situation ants do not express any marked preference for either side of the pipe. This result permits us to state that no topographical or internal references are used. Thus, the external orientating mechanisms are based either on the light direction or on a chemical trail laid down during the previous training session.

When neither of these is available, ants still ascend the left side of the pipe using the other cue.

When the light source stays in the same position and the pipe is half way round the vertical axis, a discrepancy in the direction indicated by the two cues is introduced. The results remain unchanged. On the contrary, when this last situation is inverted, no significant difference appears between the two sides. However, a slight trend toward the lighted side is still present.

Finally, when the directional information is inverted, an overwhelming majority of workers ascends the right side, resulting in a reverse image of the standard situation.

We can conclude from these results that, when moving on the vertical axis, ants not only refer to gravity, as already known, but also to chemical and preferentially to directional light.

2°) Horizontal plane

In the standard situation (Control I) ants clearly choose the end at which the food has been placed.

When no directional information is available there is no significant preference both for the initial and the final orientation. Again, as for the vertical axis, we can definitely exclude any internal or topographical cues.

The results remain unaltered when only one of the directional pieces of information is available. Ants keep on orientating to the rewarding bar using either the directional light or the chemical trail. However, we note that the orientation based on the directional light is abandoned by the majority. When the light source stays in the same place and the system is half rotated, a fairly large number of ants choose the marked bar according to chemical cues and go to the end of it. However, the preference is still significant for the rewarding bar.

Surprisingly, the results in the inverted situation are not exactly the same. A discrepancy between the initial and final orientation can be observed as already mentioned. Ants first orient according to the directional light and then switch to the chemical cues. They end their course at the end of the rewarding bar.

When the directional information is inverted, both directional cues lead ants to the bar opposite the food location. Once again, we obtain the reverse image of the Control I situation.

CONCLUSION

To sum up, this study of spatial orientation on a multidimensional system shows that on the vertical axis ants not only use gravity but also chemical and directional light with a preference for the latter. As for the horizontal orientation, both references are utilized but chemical ones seems to be more reliable in case of ambiguity between the directions indicated by the two cues. This reliability on chemical information could be explained by the fact that this cue is provided by the insect itself and that the chemical trail links the nest directly to the food source without possibility of error. However, when the chemical cues are disturbed, ants can still rely on the complementing cues as well.

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Prey Hunting and Interactions Among *Polybia occidentalis* and *Polybia diguetana* Foragers (Hymenoptera: Vespidae)

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The foraging of social wasps has been studied primarily in the Vespinae (Parrish and Fowler, 1983) and *Polistes* (Gould and Jeanne, 1984). I have determined the responses of hunting *Polybia occidentalis* and *Polybia diguetana* in Costa Rica to prey cues, and looked at factors influencing the behavior of these wasps when they encounter one another on prey.

HUNTING BEHAVIOR

I tested the responses of the wasps to visual and olfactory prey (*Bagisara repanda*, Noctuidae) cues using choice tests (methods in Raveret Richter and Jeanne, 1985; $N=15$, $\alpha=0.05$). Hunting *P. occidentalis* and *P. diguetana* foragers hovered in response to visual cues. *Polybia occidentalis* landed in response to olfactory cues. *Polybia diguetana* foragers hovered near olfactory cues paired with either controls or visual cues, but were more selective in choosing landing sites. They landed preferentially only on odor cues within 5 cm of a visual cue.

INTERACTIONS AMONG FORAGERS

I tested to see if the presence and species of wasps on a kill 1) serves as a prey location cue and 2) influences the behavior of an approaching wasp. Site-trained foragers were offered a choice between a 0.5 cm piece of caterpillar and an identical piece of caterpillar with an ethanol extract, dried posed *P. diguetana*, *P. occidentalis*, or *Polistes instabilis* placed over it.

Table 1 summarizes the results of these tests. In all cases, the wasps tended to direct their first response, hovering, at the caterpillar with the wasp posed over it. This suggests that the presence of another wasp is useful as a cue for prey location. However, the subsequent behavior of the approaching wasp differed depending upon the identity of both the approaching wasp and the dried wasp placed over the caterpillar.

Polybia occidentalis tended to land and bite where there was a dried, posed wasp of either *Polybia* species. Opportunistic observations on live wasps show that in these situations, a live *P. occidentalis* stands a chance of successfully taking a piece of prey from the other wasp. However, these wasps tended to avoid landing where a dried, posed *Polistes* was present. This large *Polistes* is capable of defending at least moderate sized prey from approaching *Polybia*.

In no case did *P. diguetana* foragers show a preference for landing with a dried, posed wasp, and in the case of a dried *P. occidentalis*, they actually avoided landing. Given the similarity in the appearance of these two *Polybia* species, such discrimination on the basis of visual cues would be remarkable; lack of olfactory cues on the dry wasp

may also be important.

The failure of *P. diguetana* to avoid landing under a dried *Polistes* was an unexpected result. Such landing was preceded by an extended bout ($\bar{X}=36$ sec.) of repeatedly touching the dried wasp. A live *Polistes* would have chased off the approaching wasp in response to such touching.

Though neither *Polybia* species landed preferentially at caterpillars under larger dried wasps, they did tend to direct their first response, hovering, at these wasps and caterpillars. Prey, usually *B. repanda* at this site, are difficult to capture, making prey theft an attractive alternative to hunting and capturing. I have observed two strategies for theft--taking all or a portion of the prey directly from another wasp, or, on large pieces of prey, waiting and stealing pieces of prey while the wasp is ferrying loads back to her nest. Wasps that do not fare well in encounters with the wasp that captured the prey would do well to adopt this latter strategy. In opportunistic observations where the wasps are not offered a choice of landing sites, I have seen both *Polybia* species do just that -- *Polybia* foragers who had discovered prey on which a larger wasp was feeding returned repeatedly to the site and, when the other wasp was back at her nest, the smaller forager landed, bit off a piece of the prey, and flew from the carcass with it.

CONCLUSIONS

Hunting *P. occidentalis* and *P. diguetana* differ in how they respond to prey related cues. Both hover in response to visual and olfactory cues. *Polybia occidentalis* also lands in response to olfactory cues; the more selective *P. diguetana* does so only if the odor is near a visual cue. When either wasp is foraging, it can use the presence of another wasp as a prey location cue. A forager's subsequent behavior depends upon the identity of the wasp on the prey.

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Polybia occidentalis forager:

Model	# trials	1st hover wasp+cp	1st hover and bite wasp+cp	1st hover cp	1st hover and bite cp
Pd	30	21	17/21***	9	6/9 NS
Po	30	21	15/21***	9	6/9 NS
Pi	20	17	3/17 ***	3	3/3 NS

Polybia diguetana forager:

model	# trials	1st hover wasp+cp	1st hover and bite wasp+cp	1st hover cp	1st hover and bite cp
Pd	20	15	8/15 NS	5	3/5 NS
Po	20	15	4/16***	4	4/4***
Pi	20	15	5/15 NS	5	4/5 NS

Table 1. Responses of *P. occidentalis* and *P. diguetana* foragers to a choice between a caterpillar with a dried, posed wasp and a lone caterpillar. Pd=*P. diguetana*, Po=*P. occidentalis*, Pi=*Polistes instabilis*, cp=caterpillar, NS=not significant, ***=significant at $\alpha=0.05$.

Recruiting Behaviour of Honey Bees as a Function of Race, Age and Quality of Chemical Conditioning Stimuli

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Honeybees recruiting behaviour upon a food source is a basic phenomenon of bee-plant relationships, leading to both apicultural production and to plant improvement through entomophilous pollination. Foraging is based on a conditioning process where food reward (nectar-pollen) is associated with different plant cues (as scent, color, shape, localisation).

Recruiting behaviour is studied in controlled conditions simulating the natural foraging situation and using an artificial flower device where a sugary solution is associated with a scent. Recruiting process is analyzed at the level of a whole colony where foragers may be individualized by coloured spots of numbered marks.

In a first step genetical abilities of honey bees to forage are studied by comparing the number of visits as a function of time upon a food source (sucrose 50 %) associated with a scent (geraniol 10^{-2} v/v) for inbred colonies (*Apis mellifica ligustica* L.) and hybrid colonies *Apis mellifica* (*ligustica* x *caucasica*) x *mellifica*. It appears that hybrid perform a much higher level of visits than inbred bees, corresponding to a better pollinating efficiency in field conditions. Following experiments were then carried out with hybrid colonies.

In a second step, distribution of foraging activity among worker bees as a function of age is studied with a colony set up with different classes of knownaged bees. Proportions of visits performed by each class of age point out that (i) foragers are at least one month old (ii) the first foragers recruited belong to the first class of age (iii) foraging is performed by a constant pool of foragers from the oldest class of age, while contribution of the other classes is low. Thus, for a constant food source, no real turnover points out among the different classes of age, as long as the pool of foragers is enough to assume colony needs.

In a third step recruiting process is observed on two food sources (sucrose 50 %) differently scented (Geraniol-limonene 10^{-2} v/v), simultaneously presented at equal distance of the hive, and regularly inverted. The first chosen scent and side are recorded for each recruited forager ; their faithfulness to the chosen scent or side is then noted along the following visits. It appears that most of foragers behave randomly versus scent but are faithful to the side. Therefore, for equally rewarded sources, a hierarchy occurs among orientation cues, spatial cues prevailing on olfactory cues.

In a fourth step, recruiting is studied according to the quality of the reward (for the most common sugars present in floral nectar : glucose-fructose-sucrose). The highest level of recruiting is obtained with sucrose solution versus fructose solution, while no recruiting occurs with glucose.

Such parameters as genetical origin, aging, orientation cues and quality of food which affect recruiting process should be taken into account for pollinating insects management.

Comb Structure, Temperature Regulation, Hoarding and Foraging Behaviour of *Apis mellifera* L. and *Apis cerana* F.

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European honeybee (*Apis mellifera* L.) has been repeatedly introduced into Asian countries over the past 100 years with varying success. Large scale importation and multiplication of this exotic species in this continent has become a controversial issue among the bee experts. Before its mass scale introduction, it is important to study the adaptation of this exotic species in its new environment and compare its biological characters with native bee species (*Apis cerana* F.). The latter species has many characters of economic and biological importance which are unexplored. The present paper is a part of the investigations conducted in this direction by our research group.

Mean volume and depth of worker comb cell of *A.mellifera* is 67 and 16 per cent greater than *A.cerana* ($P<0.01$), respectively. In the *A.cerana* comb, the number of worker brood cells ($4.04\pm0.03^*$ per sq.cm) is significantly greater ($P<0.01$) than *A.mellifera* (3.51 ± 0.01). Laboratory hoarding behaviour by worker bees of *A.mellifera* and *A.cerana* reveals that on days 1-14, *A.mellifera* hoarded (100.40 ± 1.56 mgm/bee/day) significantly ($P<0.01$) greater amount of sugar syrup than *A.cerana* (78.87 ± 2.84 mgm/bee/day). However, there were no significant differences within the colonies of *A.mellifera* or *A.cerana* in their respective hoarding behaviour.

For studies on temperature regulation, both species were kept in Ludhiana (Panjab) under the same climatic conditions (Verma, 1970). In summer at 1200 hrs, when the mean outside air temperature was 39.0°C both the species significantly ($P<0.01$) lowered their hive temperatures. When the outside air temperature exceeded 40°C , *A.cerana* colonies absconded frequently and no such absconding tendency was observed in *A.mellifera*. At temperatures above 40°C , the fanning was more regular and frequent in *A.mellifera* than *A.cerana*. Results on high and low lethal temperatures suggest that both species survived longer ($P<0.01$) at 50°C in October than in January. At both times of the year, *A.mellifera* survived high temperature longer than *A.cerana*.

Foraging behaviour of *A.cerana* and *A.mellifera* in relation to pollination of apple bloom was studied in Shimla Hills of north-west Himalayas. Worker bees of *A.cerana* started their foraging activity significantly ($P<0.01$) earlier in the morning (meantime, 0603 hrs) than *A.mellifera* (meantime 0627 hrs). In the evening, *A.mellifera* ceased its foraging activity significantly ($P<0.01$) earlier (meantime, 1855 hrs) than *A.cerana* (meantime, 1913 hrs). The duration of foraging trip of *A.mellifera* ($17.92\text{ minutes}\pm0.36$) was significantly longer ($P<0.01$) than that of *A.cerana* ($11.85\text{ minutes}\pm0.36$). In both species, nectar collectors outnumbered pollen collectors. In *A.cerana* no pollen plus nectar collectors were found, whereas in *A.mellifera* the percentage of such worker bees varied from 6 to 11 during different hours. Peak foraging activity of *A.cerana* was between 0900 to 1130 hrs. when the temperature ranged from 15.5 to 21.0°C , and that of *A.mellifera* was between 1100 to 1320 hrs. when the orchard temperature ranged from 21 to 25°C . This is remarkable from pollination point of view because by keeping both the species of honeybees in the same orchard, the duration of peak periods of foraging activity can be prolonged to ensure better pollination. The number of fibres in dorso-longitudinal, dorsoventral, pleurosternal and intersegmental muscles of *A.mellifera* were significantly greater than *A.cerana* ($P<0.01$). Similarly, diameter of flight muscles of *A.mellifera* ($201.97\mu\pm0.13$) was significantly more ($P<0.01$) than *A.cerana* ($174.90\mu\pm0.1$)

*=Standard error about the mean.

Fire Ant Foraging Behavior: a Multiplicity of Strategies and Mechanisms

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Fire ants utilize a variety of orientation mechanisms, most of which increase their foraging efficiency. Wilson (1962) studied the organization of mass foraging in fire ants and found a complex system centered around the use of trail pheromones. Over the last 20 years other fire ant orientation mechanisms have been investigated and trail pheromone work has been expanded. These are outlined below.

LIGHT ORIENTATION

It has been demonstrated that light plays a dominating role in initial trail formation in fire ants. Foraging workers randomly search for food; but once they find it they go directly back to the nest. Our studies showed that in the absence of a light source or if the light source was rotated, initial trail formation to a new food source was significantly reduced. Once a pheromone trail was established rotation of the light had no effect on the trailing workers. Fire ants use visual cues to track and orientate back to the nest after their first discovery of a food source. The most dominant visual cue is a light source, such as the sun, moon, or a street light (see Vander Meer, 1986; Vander Meer, unpublished).

TRAIL PHEROMONE ORIENTATION

See Vander Meer, R.K., Trail Pheromone section of "The fire ant sting apparatus: A case of harmonious parsimony" in this volume.

MISCELLANEOUS

In the absence of trail pheromone cues fire ants learn the route to a food source through a maze, by their ability to retain a combination of the distal-visual and kinesthetic cues required to return to the food source. Workers acting as sanitary engineers preferentially carry refuse downhill. *S. invicta* workers respond to their own nest soil in preference to soil from other conspecific colonies or non-nested soil. This may be extended to recognition of their foraging tunnels or total foraging area, thereby increasing their ability to find their way back to the correct nest. See Vander Meer (1986) for references.

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Adaptations of Myrmicine Ant Genus *Ocymyrmex* for Exploiting a Hot Arid Environment

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Members of the insectivorous ant genus *Ocymyrmex* occur in hot arid and semi-arid regions of the Ethiopian zoogeographical zone where rainfall events are rare and unpredictable. These rainfall patterns affect *Ocymyrmex* species in two important ways. First, mating and colony founding conditions are rare and temporally unpredictable. Secondly food is spatially and temporally unpredictable. *Ocymyrmex* species have evolved a suite of characteristics that facilitates their survival and reproduction in these physiologically stressful and ecologically unpredictable environments.

PROCEDURE

Field and laboratory observations were made on the foraging behaviour and social structure of ten species in southern Africa. The proportions and tasks of the different castes was determined for entire colonies and for the forager force.

RESULTS AND DISCUSSION

Colonies comprised 200-1000 ants and included one inseminated, reproductively active ergatogyne, a few alate males and many sterile workers and reproductively inactive ergatoids. The proportion of ergatoids varied considerably; means for nine species ranged from 3.5-43.6%, with minimum values of 0-27.7% and maximum values of 5.6-58.5%. Irrespective of species or time of year, all nests contained some uninseminated, reproductively inactive ergatoids. Ergatoids are therefore produced throughout the year. Reproductively inactive ergatoids adopted worker-like roles, with large numbers occurring in the forager force. It is suggested that this caste flexibility is an energetically inexpensive way of ensuring the continuous availability of young virgin ergatoids to take advantage of irregularly occurring mating and colony founding conditions.

Foragers adjusted activity levels according to the temporal abundance and availability of prey. Through the frequent use of refuges ants scavenged dead or torpid prey items, generated by intense midday heat and desiccation, at surface temperatures of up to 68°C; conditions when no other scavengers were active. On days when termites were active, ants switched to predatory behaviour and were more active during cooler conditions. *Ocymyrmex* species were usually diffuse foragers, reflecting their spatially unpredictable food resource, but a high proportion of the normal forager force as well as young virgin foragers were recruited to rewarding areas when they were located. Pheromone mediated recruitment was equally effective over a wide range of surface temperatures (29-64°C); with approximately 80% of recruits discovering the food source. Plasticity in foraging behaviour enables ants to exploit a patchy and fluctuating food resource.

Prey Items Utilized by some Neotropical *Polybia* wasps (*Hym.-Vespidae*)

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As a rule, the food of wasps consists on proteins (insects, carbohydrates (nectar and secretions of coccideans and aphideans) and water. The proteins are obtained from a number of prey species, ranging from insects (including bees and wasps) to spiders (Sakagami & Fukushima, 1957 a and b; Jeanne, 1972; Spradbery, 1973).

The present paper discusses prey items captured and utilized by four species of *Polybia* (*P. occidentalis*, *P. Paulista*, *P. sericea* and *P. ignobilis*) during their active cycles, as a contribution to knowledge of the role of these wasps in neotropical ecosystems.

MATERIAL AND METHODS

Wasps carrying prey items were collected using entomological nets and brushes, weighed, fixed in Dietrich (for 24 hs.), and preserved in 70% alcohol and examined for taxonomic identification. The mean number per hour wasps carrying prey was determined to allow estimation of weight of the load collected during their active cycle.

RESULTS

The diet of the four wasp species studied included various insect orders (5 to 10). Only *P. sericea* and *P. occidentalis* caught Arachnida individuals (Acarina and Araneae, respectively).

Although there wasn't observed a capture specificity, there was some preference for lepidopteran caterpillars. The captured material was rarely intact, but several agricultural pests were identified (e.g. *Elasmopalpus lignosellus*, *Diatraea* sp., *Spodoptera frugiperda*, *Mocis latipes*, *Pectinophora gossypiella*, *Chlosyne lacinia saundersii*, *Diabrotica speciosa*). The lack of specificity in collected material could help these species of *Polybia* to adapt to occurrence fluctuations of the prey insects during the colony development, as well as, to allow them a wider geographic distribution.

The observed flow per hour of prey items carried to the four species colonies was similar to that seen in collection samples, although most wasps that returned to the nests (60 to 90%) were transporting liquids. The weight of prey transported per trip depended on the size of the single prey carried, that, on average, was estimated be about 1/5 of the wasp's weight.

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Nesting and Foraging Behaviour of the Termite Predator *Leptogenys processionalis* (Jerdon) (*Formicidae: Ponerinae*)

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Except for a brief note on a *Leptogenys* sp. by Maschwitz(1975) there was no information on *L. processionalis*. The study was aimed to know the predatory potential of the ant, its nesting and foraging behaviour. Seventy-two colonies were observed over a period of one year at Bangalore, India.

Materials and Methods

Studies were made on the habitat, nesting and foraging behaviour. Fortnightly observations were made for a year in the study area. Population was estimated by excavating the nest, anaesthetizing with chloroform spray, and hand sorting individuals.

Results

Out of 72 nests observed, 59 were found under shade. The nest openings were more during rainy season. Ants were active even during day time in cloudy and rainy days. In summer their activity was restricted to cool hours of early morning and late evening which coincided with the termite foraging hours. The number of nests per unit area increased during wet months (20 to 21/ha) compared to dry season (9 to 16/ha).

Normally the nests were built in the existing soil cavities of varying depth (0.75 to 1.25 m) with a number of chambers. The top chambers were filled with pupae and callows, the middle with only pupae and the lower with queen, larvae and eggs. The population of a colony varied from 9,750 to 23,702, in the eleven nests that were excavated. On an average a colony consisted of 55.5 % workers, 26 % pupae, 10.3 % larvae, 1.3 % callows and 0.56 % winged males, unspecified number of eggs and one queen per nest.

The ants fed exclusively on termites with 99.95 % of their diet being only termites. The ambushing of the prey was in an organised way, triggered by a "Chirp" noise, before encircling all the foraging termites. The maximum distance travelled by foragers to the actual foraging area was 110m, and the duration of foraging lasted between 164 to 271 minutes during morning hours. The mean number of termites carried to a colony in a day varied from 25,045 to 35,204. A maximum of five termites was carried by a single ant at a time.

The ant shifted its colony from its original site to a new site any time between one and 90 days from its establishment, to a distance varying from 50 to 120m, during evening or night. Shifting of colony did not depend on the availability of termites. *Diacamma rugosum* (Ponerinae) and a red spider occasionally predated on the workers.

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Foraging in Mono- and Polydomous *Solenopsis invicta* Buren Colonies

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The unique mosaic of monodomous (monogynous) and polydomous (polygynous) colonies was studied to bring out the foraging patterns of these two apparent *Solenopsis invicta* biotypes.

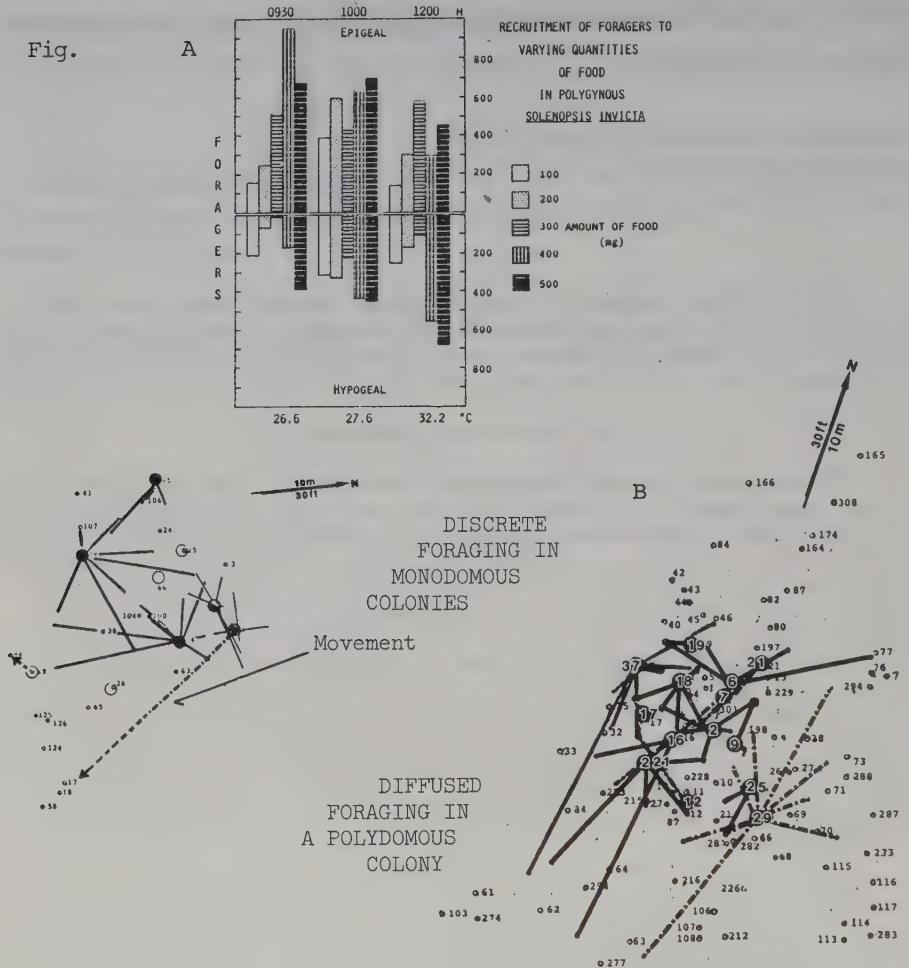
MATERIALS AND METHODS

The mono- and polydomous nests in 0.2 ha circular plots were sampled, characterized for the head widths (hw) of ants, and mapped during spring, summer and winter (see Bhatkar and Vinson, elsewhere in this volume). About 2000-5000 ants (of the estimated 200-500 thousand ants/mound) were marked using distinctive spray paints (Bhatkar and Vinson, in prep.). Under and above ground forager recruitment to 100-500 mg of cat food pellets (24% fish protein + 8.5% lipid) or to 100 mg of crystalline sucrose, marshmallows (60% sugar + 3% gelatin) and *Stomoxys calcitrans* (stable fly) pupae was studied in a Texas pasture habitat. The baits were placed at 1.5 m equidistant stations in concentric circles to define the extent of foraging by nest inhabitants.

RESULTS

An equilibrium is maintained by the foragers in recruiting to above and underground food baits (Fig A). The nestmates are recruited along chemical trails from apparently the same hypogeic foraging tunnels. There was a direct correlation of forager traffic to the amount of food during the first 30 min, which turned suboptimal during the next 30 min the food was replaced. When no food was placed (10-1030 h) during the next 30 min, followed by 2 rounds of 30 min food placement (1030-11 and 11-1130 h), monogyne foragers lingered at the food stations and quickly exploited the subsequent food placements, while the polygyne foragers returned in initial numbers with a 30 min delay (Fig A, 12h). The polygyne foragers (0.6-1.3 mm hw) communicate with nestmates and sweep the resource at a faster rate through a massive (in thousands) recruitment. The monogyne foragers (0.6-1.5 mm hw) are persistent at a local resource. Efficient food handling, such as transporting various sizes of sugar crystals, cutting gelatinous marshmallows or stable fly pupae, seems to be achieved through a bimodal recruitment: peaks between 0.6-0.85 and around 0.85-0.95 mm hw in monogyne, and between 0.55-0.75 and 0.85-0.90 mm hw in poly-

Fig.



gyne foragers. A similar tendency is observed in *S. geminata* and possibly other polymorphic species. Thus, the production of large size (major) workers, found in monogynous colony, seems to be forfeited in the polygynous colony where a large number of media size workers are dispersed over an extended area as a supercolony. Both the systems seem to be adaptive. The foragers recruit from several adjacent nests to the common food stations in a polydomous colony. On the contrary, the monogyne foragers monopolize their food stations and the colonies show seasonal (recruitment in 100s/ station in summer) and spatial fluctuation in foraging. Having depleted an area, the colonies seem to periodically relocate themselves to inhabit unoccupied mounds 1-30 m away or move to an underexploited area (Fig B).

SYMPOSIUM

The Role of Competition in Colony Organization

Organizer: Hayo H.W. Velthuis

Introduction

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In social insect colonies individuals cooperate and compete. The cooperation is visible if we keep distance. If we take a closer look, however, the colony consists of animals having conflicting interests and we find that they are competing for their chances. Those aspects we interpreted as cooperation are, at the same time, the net result of the antagonistic tendencies within the colony.

We owe much to Hamilton and to those who continued along his lines of theoretical approach. They showed that social forms are bound to certain evolutionary limitations, and because of that we see repeatedly convergent evolution in distant groups. The theory is concerned with evolutionary processes which take place on quite a different time scale than those of the individuals or the colonies we observe. The theory is concerned with averages, produced by a combination of often conflicting regulatory mechanisms. The direct transfer of the rules discovered by sociobiological theory to the level of a single colony or individual, therefore, seems not permissible. At this lower level we perceive the variation around the mean, a variation that is not included in these theoretical rules. The degree of variation is probably not without a meaning. The shift in time scale, therefore, is not merely a shift from functional to causal aspects.

Let us, for instance, consider the reproductive biology of the honeybee. Of course, since this insect needs outbreeding, different colonies should synchronize the emission of sexual forms. But there is no need to synchronize the production of males and queens within a single colony. Indeed, the mechanisms leading to the production of these forms are independent. As long as the queen is dominant the colony will not swarm and only males will be produced. The better the queen, the longer she stays, the higher the production of males. If the queen fails to control the workers queen rearing starts, leading to queen replacement or swarming. The short-range regulation of reproduction can be interpreted as a kind of parent-offspring conflict.

The observation that so many processes at the population or colony level are not organized in a straightforward way but are the result of complex intertwinings of separate processes in its constituting members might indicate a long evolutionary history of such processes. One may also pose the question whether there could be an advantage to this complexity.

Evolution is always the result of competition. I do not believe in a single mutation that makes a female an altruist and thereby a worker. Instead, I suppose that, time and again, competitive interaction patterns have been moulded, bringing about increasingly complex systems. Whatever the specific evolution might have been, however, through the inclusive fitness component the loser in classical competition has become a winner of a kind instead of a slave.

Whenever the regulations at colony level have the character of dynamic equilibria we should not expect the outcome to be the same for all

colonies. If the population is best adapted when male and female sexuals appear at a certain time and in a certain ratio, not all colonies necessarily should produce them in that average manner. If we turn again to the honeybee I could imagine a colony whose queen is exceptionally strong. Such a colony can produce so many males that they dominate the market when it comes to mating. In contrast, even a strong colony will not produce more than 2-4 swarms. This could be said in another way: in their male production individual colonies follow the r-strategy: many cheap individuals produced at low risk will lead to an occasional large dissemination of genetic material. In their female reproduction line colonies are K-strategists: a low rate of increase but every queen will be surrounded by the utmost guarantees. Within the colony the two possible ways of reproduction are based on competing regulatory mechanisms, queen dominance and worker initiation of queen rearing. At the population level the average gain made by each of these strategies is, of course, in equilibrium. At colony level, however, reproduction is rather variable, being determined by many interfering factors.

In this symposium several examples of competition are brought together. We will deal with its effects on quite different time scales. Attention will be given to differences among individuals and how this influences aspects of the colony they live in; to modifications that make populations inhabiting different geographical areas to be quite distinct. The question arises whether such features are adaptations or just random occurrences. Also, in some contributions, competition and its effects are shown in full evolutionary perspective, in that competition apparently led to complexity of interaction patterns and to several ways in which reproductive success can be achieved. It is no novel statement that decisions taken in the evolutionary past limit the evolutionary options open for a further development. In Kukuk's paper, however, the wide range such consequences may have are beautifully exposed for Halictid evolution.

Fighting Males in the Ant Genus *Cardiocondyla*

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Cardiocondyla wroughtonii (Forel) colonies produce two types of males: a winged male and a wingless, workerlike (or ergatoid) male. Nests are often polygynous but typically contain no more than one ergatoid male which mates with the young queens produced there (Lupo and Galil, 1985, Stuart, unpublished). The ergatoid males are especially noteworthy in that they have long, sickle-shaped, toothless mandibles, quite different from the multi-toothed mandibles characteristic of conspecific females and most congeneric males (see Kugler, 1983). These unusual mandibles led to speculation that these males may fight as a form of intrasexual reproductive competition (Hamilton, 1979). Our observations confirm this speculation.

METHODS

C. wroughtonii colonies were collected in Florida from cavities in *Catalpa* twigs, pine twigs, and pine cones. In the laboratory, colonies were cultured in plastic nests and foraging dishes with transparent lids (see Stuart, 1985). Colonies proliferated rapidly and were often split up to form additional colonies. Most of the observations reported here were made during a five-week period and involved nine large colonies which contained 3–21 dealate queens (\bar{x} = 8.1, SD = 5.75) and 145–700 workers (\bar{x} = 376.1, SD = 200.20). A number of smaller colonies (i.e. 20–30 adults and brood) were also observed.

RESULTS AND DISCUSSION

Fights between ergatoid males occurred when multiple ergatoid males eclosed in the same nest, and when colonies which had been inhabiting multiple nests in the same foraging dish (each with their own ergatoid male) fused into a single nest. A total of 26 lethal fights between ergatoid males were directly observed, and many more could be inferred from the sudden disappearance of ergatoid male pupae at the time of eclosion, and the discovery of ergatoid male bodies and body parts.

In smaller colonies, a resident ergatoid male typically discovers and attacks a young male soon after it begins to eclose, before it is able to attack; and the young male is quickly killed, its soft body dismembered, and fed to larvae or removed from the nest. In large colonies, an eclosing ergatoid male often becomes completely free of its pupal integument and begins wandering about the nest before it encounters the resident male. Fights between older males often last several hours and either male may emerge victorious.

Ergatoid males display a stereotyped fighting behaviour. When the males discover one another they become extremely active, antennate one another intensively, climb over and around one another, entwine their legs, and roll about in grappling fashion. During this process, the males bite at one another sporadically, but this results in no apparent

injury. Eventually, one male secures a firm grip on the neck, alitrunk, or petiolar region of the other, generally from a dorsal or near dorsal perspective, and the victim is virtually helpless. The mandibles of these males are probably incapable of causing dismemberment, and never appear to pierce the exoskeleton or sever any major body parts, but the fighting grip of these males may be held for several hours, and this appears to cause death, perhaps by constriction. A dorsally applied grip to the neck is especially common.

Once a firm grip is established, the aggressor male begins to beat the victim with its prothoracic legs in a rapid, alternating, and fairly continuous manner; and he occasionally applies his gaster tip to various parts of the victim's body. Generally, other colony members show little interest in the fighting males, but occasionally antennate, lick, and bite them. Indeed, worker bites appear solely responsible for the dismemberment of victim males during fights. Occasionally, aggressor males also lose appendages in this way. The relative importance of dismemberment in the death of the victim is unknown, but by the time the aggressor releases its grip, the victim has generally lost a number of appendages and appears near death. Aggressor males may actually induce worker aggression, perhaps through a secretion applied from the gaster tip. The beating movements of the aggressor's legs may serve to spread this secretion, or to keep these appendages out of the grasp of biting workers.

In the laboratory, winged males often eclose in the same nest as ergatoid males, but they do not become involved in fights and generally show little interest in the young queens. Nonetheless, in experimental nests, both types of males proved capable of inseminating young queens; and queens from both types of matings produced apparently normal worker offspring. In nature, winged males may disperse from their parental nests and pursue an alternative reproductive strategy. Occasionally, multiple ergatoid males eclose and persist together in the same nest. However, this appears to be relatively rare (see Lupo and Galil, 1985). Multiple ergatoid males have also been reported in nests of other Cardiocondyla species (see Kugler, 1983).

Our observations indicate that fighting among C. wroughtonii ergatoid males is probably the major reason that nests typically contain only one ergatoid male. This fighting appears to be a manifestation of intrasexual reproductive competition and, by fighting, victorious males may well secure exclusive mating rights in their parental nests. This is the first report of lethal fighting among males in the ant genus Cardiocondyla, and the second observation of this phenomenon in ants (see Hamilton, 1979).

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Competition Over Oviposition in Subtropical and Temperate Populations of the Social Sweat Bee *Halictus ligatus*

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INTRODUCTION

Increased reproductive dominance of the queen over her workers may be considered as the major feature of social evolution in the Hymenoptera. Unfortunately, most taxa that contain social species do so to the exclusion of solitary forms (with the exception of social parasites). This is why the study of halictine bees is so important - single genera may contain both solitary and social species with colony sizes often exceeding several hundred individuals. Clearly these organisms are excellent candidates for addressing the evolution of a reproductive division of labour.

In this paper, I present data on competition over direct reproduction in two populations of the social sweat bee *Halictus ligatus*. In one population (S. Ontario), the single worker brood may be responsible for laying most of the haploid eggs (Packer, 1986, Can. J. Zool.). In the other (Florida Keys), the workers contribute such a large proportion of the eggs that the queen's reproductive superiority rests upon her greater longevity. This latter population is continuously brooded and multivoltine (Packer and Knerer, 1986, Behav. Ecol. and Sociobiol.).

METHODS

The S. Ontario population was studied in detail in 1984, over 130 nests were excavated. The Florida population was observed intermittently during the years 1981 - 1984 and 56 of the metre deep nests were completely excavated.

Female bees were dissected and their reproductive condition noted in detail. Each ovariole (of which there are 6) was assessed as to the fraction of a fully developed oocyte that it contained (halictines typically undergo ovarian development of only one egg per ovariole at a time). Categories of 0, 1/4, 1/2, 3/4 and 1 were used. Additionally, the contents were classified as developing or being resorbed, this latter condition being indicated by discolouration or a misshapen appearance. That resorbing ovariole contents do in fact represent resorbing eggs was verified by experiment. Oviposition success was estimated for queen and worker castes separately by subtracting the number of resorbing fully developed oocytes from the number of healthy, fully developed oocytes found within the bees. Oophagy was detected by examining the gut contents of bees.

Influences on ovarian development were analysed by multiple linear regression. Dependent variables were the sum of developing or resorbing oocytes or the total of these two figures, for individual queens or workers. Independent variables were a range of nest-related factors such as the age of the nest, number of workers, queen-worker size dimorphism and the various ovarian scores for the remaining nestmates.

RESULTS

A summary of the multiple regression analyses is as follows. For the Ontario population, positive relationships were found between queen and worker ovarian scores, ovarian resorption in queens being more closely related to the number of workers in the nest. In the Florida population, negative relationships between queen and worker ovarian development were obtained. Slight evidence for mutual inhibition over ovarian development amongst the workers was also obtained.

In the Ontario population workers appear to be responsible for most of the male production whereas in Florida workers produce about 90 % of all eggs. With a sex ratio of 0.8:1 females to males it seems that workers must be capable of laying diploid eggs.

DISCUSSION

Strong mutual inhibition over ovarian development seems to occur amongst queens and workers in the Florida population. In contrast, positive relationships were found amongst queen and worker ovarian scores in Ontario. The latter result probably stems from any inhibitory influences being swamped by more powerful forces that affect all members of a colony simultaneously - the weather being a prime candidate in this instance. Oophagy did occur in both queens and workers in both populations but seemed to depress the direct productivity of both castes about equally. This indicates that there is indeed some competition over oviposition even in the Ontario nests.

In both populations, queens have to resorb more oocytes when the worker population is larger. In Ontario, the number of workers in the nests remain small throughout the summer, this resulting from a slow rate of provisioning by the queen in spring. In Florida, nest populations exceed 20 individuals. However, the slope of colony productivity against number of workers is great enough to more than compensate for the queen's reduced direct reproductive success.

In Ontario, workers are probably restricted to laying haploid eggs - most are unmated at the time when they have the most ovarian development. The Florida population is continuously brooded and multivoltine, this results in males being active at all times of the year, and over 50 % of the workers do mate. This explains the deduction that workers appear to lay both haploid and diploid eggs.

This study concerned two populations of a social sweat bee with very different degrees of queen control. However, in both populations the genetic interests of the queen seem to be met. Thus, even the earlier stages of social evolution, involving a great deal of reproductive competition, may be in the queens best interests.

Alternative Social Structures in Halictine Bees

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A wide range of diversity in social organization is found in Halictine bees. Communal behavior has arisen repeatedly in certain lineages while eusocial (and semisocial) behavior has arisen repeatedly in others. This suggests that there are two alternative, evolutionary solutions structuring group life in halictine bees. Direct evidence in the eusocial *Dialictus zephyrus* has shown that social organization, including caste determination, occurs due to behavioral interactions among adult females within nests (c.f. Buckle, 1985). We investigated the possibility that the arena within which these interactions occurs, the nest itself, influences social organization.

MATERIALS AND METHODS

Data were obtained from the extensive literature on the nest architecture and social behavior of halictine bees. The Coefficient of Variation (CV) in head width and wing length was calculated for solitary ($n=6$), communal ($n=2$) and eusocial ($n=20$) species. Obligate Joint Use Region (OJUR) of the nest was calculated for each species ($n=66$) by dividing the length of the burrow that must be used by all the females in the nest by the total length of the nest burrow. The ratio of lateral length to brood cell length (L/C) was also determined by measurement of the published nest diagrams (a "lateral" is a narrow tunnel leading directly from a main burrow to a brood cell; see Sakagami and Eickwort, 1979).

RESULTS

There are no differences in the CV of communal and eusocial species suggesting that variance in reproductive potential is the same for both. The OJUR of solitary species ($n=17$) is significantly larger than that of both communal ($n=15$) and eusocial species ($n=32$); moreover, the OJUR of communal species is significantly larger than that of eusocial species suggesting that individuals within communal nests are not obligated to interact with one another as frequently as individuals in eusocial nests. In species making the transition from solitary to group life a nest with a low OJUR would allow potential subordinates to avoid contact with potentially dominant nestmates without leaving the nest.

The primitive (L/C) ratio as determined from 12 species of solitary Nomioidini and Dufoureae is 2. Both solitary and communal species

($n=9$) in the Agapostemon complex of genera have L/C ratios that exceed this value. This suggests that communal behavior in this group arose in the context of nests with long laterals. An overall trend is apparent, solitary species are found in both primitive and more advanced types of nests but no eusocial species are found in primitive nests. This suggests that advanced nest architecture preceded the evolution of eusociality. The Australian halictines, hoerbrt, are nearly all communal and appear to have varied nest architectures ranging from primitive type to moderately advanced types. Perhaps communal behavior evolved in the context of both primitive and moderately advanced nest in halictine bees.

DISCUSSION

The arena within which adult females interact may be of major importance in determining social organization. A nest that allows individuals the chance to avoid contact with nestmates and still gain the advantages of group life sets the stage for the evolution of communal behavior. In contrast, a nest that forces continuous contact among related individuals sets the stage for the evolution of eusociality.

Finally, why are communal and eusocial species found in different lineages? Hamilton's rule states that the cost to an altruist in lost individual fitness must be less than the gain for the altruist through inclusive fitness. During the initial steps in the evolution of eusociality in a solitary species high degrees of relatedness and the high risk associated with nest initiation (only about 20% of spring foundresses rear any brood) influence the cost benefit ratio. In communal species females in the same nest may not be highly related as in solitary species, and they are not compelled to risk colony initiation. Therefore, due to lowered relatedness among nestmates and higher costs in terms of individual fitness for altruists, communal species are less likely to evolve eusociality than solitary species.

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Reproductive Strategies of Colonies of *Bombus terrestris*

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On population level the sex ratio of bumblebees is highly male biased. This is inconsistent with theoretical considerations based on parent offspring conflict, leading to a prediction of 1:1 till 1:3 in favour of females. On colony level a great variability in the sex ratio and colony size is observed. Which proximate factors cause the inter-colonial variation in the sex ratio and in colony size?

Three moments in the development of each colony are of importance: 1. the start of laying diploid eggs by the queen from which workers emerge; 2. the beginning of egg laying by workers, egg eating or robbery and mutual aggression between queen and workers; we will call this the competition on point; 3., in between, the moment at which the queen shifts from laying diploid eggs to the laying of haploid, male producing eggs; because of the rather sudden occurrence we will call this the switch point.

Associated with the production of males is the production of queens. Male production was considered to be a trigger for queen rearing (Röseler 1974, Pomeroy and Plowright 1982). In *B. terrestris* the number of queens reared by a colony depends on 3 factors: the number of diploid eggs available around the switch point, the degree of inhibition by the queen and the larva/worker ratio. In the process of colony development the timing of the switch point and of the competition point occur to be prominent factors determining the investment in workers, males and in queens and also, as we will see, in the sex ratio.

The timing of the switch point, of the competition point and the consequence there of, was studied in for 26 *B. terrestris* colonies. They were reared from queens that were caught in the surroundings of Utrecht at about the end of March in the years 1982-1985. We will attempt to understand the causes and the functions of the high variability between colonies in the production of either sex and their sex ratio.

We measured the timing of the competition point from the moment the first workers emerge till the first features of the competition point. Little variation occurs in the moment of the competition point between colonies. On average the competition point occurs on day 31 of the eusocial phase, independent of external factors. The same holds for *B. terrestris sassaricus* colonies which were reared from queens caught in Sardegna during the month October. In between colony initiation and the competition point lies the switch point. The timing of the switch point scatter in a more or less bimodal distribution. In one group of colonies, called early, the queen switches on average after 10 days of the eusocial phase. In the other group, called late, this happens after 23 days. Till now no demographic data or ethological data give any indication about the cause of the switch point. Comparison between the demographic data of the early and late switching colonies reveals no difference between the groups in their solitary phases. In addition, the position of the switch point is independent from that of the competition

point. This indicates that the laying of haploid eggs by the queen does not trigger oogenesis or behavioural processes in the workers. Also the number of workers in the colony and density effects can be excluded. We believe that the competition point must be explained by an ontogenetic programming in the queen. She will lose her dominance, maybe expressed by changes in her pheromonal output.

The total number of workers, produced on average in the two types of colonies differ significantly because of the so different position of the switch point in the equal patterns of colony development. In the late colonies about twice as much workers are produced as in the early colonies. This becomes apparently only once the competition point has been passed because they emerge after this competition point. In the early colonies only the first worker group is present at the switch point. In the late colonies the second batch has emerged in the meantime. Because the rate of egg production is the same for both types, the number of workers present on the competition point will be the same, about 80 workers. Also the total number of males, produced by the queen differ considerably. This due to the so different switch points and the similar competition point. The early switching colonies produce more than twice as much males as the late colonies. It is remarkable that the total number of queens also differ significantly. In the early switching colonies only 5 out of 11 colonies rear some queens, on average 10 queens per colony. In most of the colonies the queens originated from diploid eggs incidentally laid among haploid eggs. In the late switching colonies 9 out of 10 reared queens and in the colonies of B. terrestris sassaricus 4 out of 5 did so. On average more than 50 queens per colony are produced. Partly the queens originate from eggs laid up till 7 days before the switch point; partly from diploid eggs laid among haploid eggs. For the late colonies it can be said that diploid eggs laid after the switch point will always result in queens. This is not valid for the early colonies those diploid eggs that are laid up to a few days after the switch point will become workers.

The investment in sexuals in grams per colony is the same for both types of colonies, since queens are about twice as heavy as males. This means that the small colonies, having a low number of workers, and that exclusively produce males cannot be considered as unsuccessful. They apparently have a different reproductive strategy. In our example the two strategies occur in an almost 1:1 ratio. We can imagine that males of the early switching colonies monopolize the matings by their early appearance in the field. The reproductive success, after mating, of the early colonies will be the same of that of the late colonies then. On average the late switching colonies obey to the prediction of the kin selection theory in that they produce a 1:1 sex ratio. The early colonies exploit the competition among males. Altogether, the occurrence of this parasite reproductive strategy of the early switching colonies could explain the 4:1 male biased sex ratio of bumblebees.

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An Ethological Analysis of Intra-Colonial Competition in Relation to Reproductive Dominance in the Stingless Bee *Melipona favosa*

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The inter- and intra-specific variation of laying worker behaviour is of special interest for behavioural studies of intranidal competition of stingless bees. In queenright *Melipona* colonies, laying workers commonly oviposit in cells provisioned with larval food prior to the queen. Such worker eggs are always eaten by the queen as soon as the worker withdraws from the cell immediately after her oviposition. No competition is reflected in the motor pattern of the interactions, and overt aggression is absent.

COMPETITION AND TROPHALLAXIS

Trophallaxis occurs frequently. However, there are no spontaneous offerings in the brood nest. Here, all transfers are preceded by soliciting by one of the participants. The trophallactic food is unevenly distributed. Workers participating in cell provisioning are dominant: they not only receive more food, but their solicitations are also more significantly rewarded. Similarly, all queen-worker trophallaxis interactions are initiated by solicitations. However, the queen's frequent solicitations are hardly ever rewarded. In *M. favosa* 509 solicitations took place during 862 min. of observation (incl. 59 min POP behaviour). Only nine (1.8 %) of these were followed by a food transfer.

COMPETITION AND LAYING WORKER BEHAVIOUR

Competition between workers is especially evident in queenless colonies where laying workers compete for ovipositions. Such competition however rarely occurs in queenright colonies. Queenless laying workers (QLW's) differ from regular queenright laying workers (QRW's) in the following important respects:

- They are important participants in the cell provisioning process. They often trigger the provisioning by a first discharge. QRW's are, at most, minor provisioners of the cell in which they oviposit.
- They perform many cell inspections and other cell directed behaviours during the provisioning phase.
- They always operculate the cell in which they have laid. QRW's always withdraw immediately, allowing for queen oophagy.
- They demonstrate competitive behaviour. Workers that have just finished laying and have started operculation are pushed off and pulled from the cell. Winners re-open partly closed cells, eat the loser's egg and replace this by ovipositing themselves, whereupon operculation recommences, etc.
- They release eggs that are similar to queen eggs, whereas QRW eggs have a deficient morphology.
- They release more eggs (up to 10 eggs per laying worker in *M. favosa*; whereas QRW's lay only 1-2 eggs)

-- The operculum takes less time in QLV colonies.

COMPETITION AND QUEEN-WORKER INTERACTIONS

The queen performs "dominance behaviour", suppressing workers physically by stepping on their head and thorax. The typical ambivalent behaviours of court workers are characterized by a conflict between attack and escape in these bees. The workers that most actively participate in such court acts are on the same day also most active in cell directed behaviours and in addition have ripe oocytes. This conflict behaviour of workers, which still reflects competition between queen and potential layers, has evolved into a communicative function ("ritualization"). The resting queen is activated by the behaviour of the court workers.

In conclusion, competition is an important factor in stingless bee biology. Workers compete in the nest for food and, under certain conditions, for ovipositions. The specific motor patterns of the queen-worker co-actions still reflect the aggressive origin of this behaviour. During the evolution of stingless bee sociality these agonistic interactions have evolved into communicative behaviour. The typical rhythmicity of oviposition behaviour of stingless bees is based on the cyclic occurrence of communicative court behaviour.

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Activity of Queens and Workers in Artificial Glass-Framed Soil Nests

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Several authors. Spangler & Manley (1978), on *Dasymutilla*; Ross (1983) on *Vespula*; Neilsen (1972, 1981) on *Lasius alienus* and Gordon (1983) on *Pogonomyrmex badius*, have reported that social activities inside the nest were influenced by temperature and light intensity. In *Myrmica rubra* L., it has already been suggested (Evesham 1984, 1985) that daylength affects ant activity and their distribution within a nest. This paper describes a preliminary investigation into the effects of environmental stimuli on colonies of the polygynous ant *M. rubra*.

Materials and Methods

Three colonies containing 1500 workers each and either 5, 10 or 15 marked queens were maintained in artificial soil nests (Evesham 1984) for one year. During that time, the activities of individual queens in and on the nest were recorded, for each nest, for an average of 467 hours. Two continuous observations, each for 24 hours, were carried out on one colony.

Results

Queens from each colony scored points according to the distance moved within the nest and the results are shown in Table 1. According to ANOVA, the length of the photoperiod significantly ($P < 0.001$) affected queen movements. Queens were active and appeared on the surface of the nest in spring. Activity declined as photoperiod increased. On a diurnal basis (Fig. 1), a peak of activity occurred at 2300 h and increased just after the light came on in the morning and before the light went off in the evening, remaining low around midday.

Fig. 1. Diurnal average of queen activity in and on the nest of one colony.

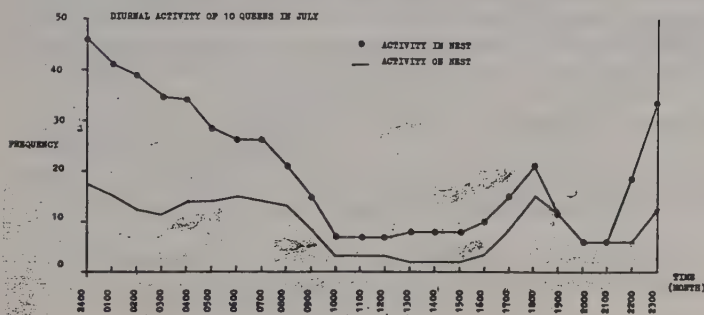


Table 1. Monthly average of queen activity in and on the nests of three different colonies.

8 QUEENS			
DAYLENGTH	MONTH	ACTIVITY IN NEST	ACTIVITY ON NEST
10 h	Feb.	244/24 = 10.167	47/24 = 1.958
12	Mar.	345/34 = 10.470	13/34 = 0.382
14	Apr.	454/63 = 7.210	25/63 = 0.397
16	May	133/22 = 6.045	5/22 = 0.227
18	Jun.	153/39 = 3.923	49/39 = 1.256
16	Jul.	88/13 = 6.769	0
14	Aug.	66/22 = 3.000	13/22 = 0.591
10 QUEENS			
12 h	Mar.	122/12 = 9.333	11/12 = 0.917
14	Apr.	204/18 = 11.333	22/18 = 1.222
16	May	347/28 = 12.393	42/28 = 1.500
18	Jun.	4/2 = 2.000	1/2 = 0.500
16	Jul.	150/20 = 7.500	10/20 = 0.500
14	Aug.	153/13 = 11.769	32/13 = 2.452
15 QUEENS			
16 h	Jul.	243/15 = 16.200	27/15 = 1.800
14	Aug.	543/26 = 20.885	12/26 = 0.462
12	Sep.	37/6 = 6.167	2/6 = 0.333
10	Oct.	123/21 = 5.857	1/21 = 0.050
8	Nov.	87/9 = 9.667	4/9 = 0.444

Discussion

In *M. rubra*, it has already been suggested (Evesham 1984, 1985), but not experimentally proved, that a peak of activity occurred at a time equidistant between sunrise and sunset. A period of twilight was not simulated in the lab., so it is possible that *Myrmica* wait until a certain light intensity is reached before becoming active. The ants were possibly affected by thermoperiods generated by the lamp providing the light source.

Is it the ants foraging on the nest surface who assess the seasons by perceiving photoperiods and temperature and then communicate their information to other members of the colony? In *Myrmica*, Smeeton (1983) suggested that since a colony was not continuously exposed to daylight due to their

occupation underground, their perception of photoperiods must become difficult without a second cue of temperature. Perhaps some biological clock is at work. In these experiments, the queens were active on the surface before the workers, so the queens may interpret the cues and initiate the colony cycle.

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Queen Competition in Polygynous Societies and Other Implications of Polygyny in the Argentine Ant

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Several works showed that in polygynous societies of social insects, the fecundity of the queens is inversely proportional to the queen number in the nest. In the Argentine ant, two hypotheses were proposed to explain the lower weight and fecundity of queens in polygynous societies (Keller and Cherix, 1984):

- An inhibition of fecundity between queens resulting from a reproductive competition.
- Social perturbations resulting from the presence of several queens in the same nest.

In order to investigate the second hypothesis, we studied in the Argentine and (*Iridomyrmex humilis* (Mayr)) the influence of polygyny on the aggregative power of the queens toward the workers.

RESULTS

Queens reared 3 months in monogynous and polygynous societies were submitted to an aggregative test similar to the one of Coglitore and Cammaerts (1981). Fig. 1 shows that queens in monogynous societies have a mean aggregative power higher than queens in polygynous societies.

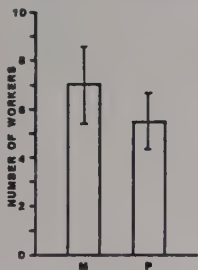


Fig. 1. Mean aggregative power of queens in monogynous societies (M)=(7.04 \pm 1.63) (\bar{X} \pm SD) and queens in polygynous societies (P)=(5.50 \pm 1.17). (t-test: t=3.89; df=50; P<0.001).

In the Argentine ant, workers get nearly all food from the workers (Markin, 1970). A lower aggregative power will then decrease the amount of food they get and could then explain their lower weight and fecundity. These data and the fact that in polygynous societies no hierarchy between queens resulting from a competition could be detected (Keller and Cherix, 1984) seem to confirm the second hypothesis.

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SYMPOSIUM

Keeping Bees in Flight Rooms - Behavioural Aspects and Techniques

Organizer: Christian Czoppelt

Introduction

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A symposium on the technology of keeping bee colonies in flight rooms was initiated for the first time in 1976 at Gießen (FRG) (Ruttner and Koeniger, 1977). Ten years later, at the 10th International Congress of IUSSI at Martinsried, a symposium on the same subject could again be organized.

The present flight room technique conforms to the system worked out by van Praagh (1972), and has continuously been perfected till now. Modern flight rooms are creating artificial and limited biotopes with a separately regulated climate simulating outdoor conditions.

Meanwhile, the behaviour of bee colonies is well studied in such artificial environments. But although important progress has been made in the field of technology, flight room colonies are not able so far to maintain their biological features on the level of outdoor colonies.

Only a few unsolved problems in connection with the present flight room technique shall be mentioned here: No increase in the population of a colony occurs during a longer period of time. Stagnation or even decrease of the population prevent swarming activity. In addition, breeding of males was never observed.

Studies on the behaviour of bee colonies have directly approached the problems of food supply: Is it possible to optimize food supply in such a manner, that it can really mimic fresh food from the outside? Which are the factors responsible for an increase of flight and brood activity? Can physiological changes be induced by special food supply which also influence social organization and structure? Will swarming and breeding of males be possible at all under the conditions of restricted artificial biotopes? All these aspects are in the focus of interest and are waiting for a solution in the next future.

The symposium dealt first with problems of food supply and its influence on brood development and foraging behaviour of honey bees (*Apis mellifera*). Supply of pollen and sugar - both offered as basic food - seemed to be sufficient for normal brood development. Besides the total amount of collected pollen, the continuity of pollen supply is also important for colony development. The obvious decrease in brood development is attributed to the lack of nurse bees and also to the restriction of flight space. Pollen-collecting behaviour varies according to the conditions of the artificial climate. Increase of the relative humidity up to 80 - 90 % stimulated foraging activity, whereas a low rela-

tive humidity of 60 - 70 % led to a decrease of foraging activity.

By means of time training experiments it was shown that South African honey bees (Apis mellifera capensis) developed anticipating activities in an artificial environment. The observed anticipation of feeding time is considered to represent the phase angle difference between the periodical onset of food presentation and an entrained circadian rhythm.

Keeping of Brazilian stingless bees (Scaptotrigona postica depilis) in a flight room is generally possible without any problems. This was demonstrated by keeping such colonies for over 12 years in sequence. Provision of pollen and syrup for collection was sufficient to keep the colonies in good condition throughout the winter season. In contrast to honey bees, production of queens and males, respectively, proceeds normally.

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Is Supply of Pollen and Sugar Sufficient for Brood Development of Honey Bee Colonies in a Flight Room?

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Studies of many years have shown that from the beginning of flight room period development of bee colonies in a flight cage followed natural realities without any symptoms of restrictions on brood or behaviour. Later a continuous decline of all developmental features of a colony occurred. Therefore the question arised whether the offered food was sufficient for brood development or provision of additional nutrients or improvement of environmental conditions could prevent decline of a colony.

MATERIALS AND METHODS

A bee colony (*Apis mellifera* L.) with originally 10 000 adults was kept in a flight room through whole winter season from October to March. Dimensions and size of the flight cage and also ascertainment of brood area has been described (Czoppelt *et al.*, 1980). Day (15 h), dawn (1 h) and night (8 h) were simulated and were following the rhythm of a summer day and night, resp. The room was kept at 45 ± 5 % rel. humidity and at day and night temperatures of 24 ± 1 and 19 ± 1 °C, resp. The basic food which consisted only of pollen, sugar solution and water was daily provided for collection ad libidum.

RESULTS

Pollen freshly grinded (0.05 mm Ø) was easily collected by workers at a rel. humidity of 45 %. Increase of humidity up to 50 % and more caused sticking of pollen without any chance of being collected. The smaller the pollen grains were the higher was their readiness of sticking due to their high hygroscopic nature. In November the amount of collected pollen was about 50 % of the initially offered one. Later, in January, the level of daily consumption decreased to an average of 40 % and at least to 25 %.

Sugar solution was offered as a mixture of sugar (50-65 %) and honey (5-10 %). Honey was for taste and attraction which could be increased by addition of different essential oils. In the beginning sugar solution was collected very intensively with 1600 ml/week. Then after two weeks consumption decreased very fast to a rather low but for the following period to a very constant level of about 200 ml/week.

Consumption of water was also high in November with 600 to 800 ml/week and low in December at a level of about 400 ml/week. In January water was collected not at all. Instead

of it provision of salt solution, either as Ringer solution (pH 7.0) or as mixture with ammonia (pH 8.5) was accepted by the workers.

Colony weight decreased constantly from about 45 to 30 kg during the whole season.

Mortality of adults was even high at beginning of season with 2000 - 3000 dead bees per week. At season's end only 500 dead bees were found which correspond to a rate of 25 %, although no disease was noted.

An increase of total brood area from 900 to 1300 cm² in November was followed by a few weeks constant brood level of about 900 cm² and at least by a continuous drop down to half of the original area in March. A comparison of area of open and sealed brood cells has shown that the first was predominating the latter one. A fine correlation between both areas was found only in the first three weeks of observation period. Later subsequently more eggs were laid by the queen than larvae could be reared. Obviously eggs were removed in consequence of a lack of workers which have to provide for the brood. From 8000 workers in November at least about 2000 workers in March have remained. This reduction to a smaller colony was necessary because of the small number of nurse bees which are not able to provide all young larvae sufficiently with food.

The decrease in number of bees led to a critical situation: Removing of eggs, limited production of adults, less individuals inside the hive to warm and provide for the brood, reduction of brood nest to a low level which, however, guarantees even sufficient maintenance of the colony. At least the size of brood nest was reduced from seven to two combs.

Concerning environmental conditions only low rel. humidity of 45 % could be objected. 80 % and more are necessary for a sufficient maintenance of a colony (van Praagh, 1975). Only higher consumption of water could counteract a low rel. humidity.

During the whole season supply of basic food seemed to be sufficient for a normal development of a colony. Additional nutrients did not improve brood quantity. Decrease in consumption of provided food (pollen and sugar) - due to the lack of workers - was substituted by increasing intake of stored food in the combs in February and March. The size of area of stored pollen and honey in the brood combs fluctuated weekly.

Responsible for this situation seemed to be neither the kind of nutrition nor the quality of food, but the restriction of flight space which induced reduction of colony strength to a minimum size necessary for surviving.

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Pollen Collecting Behaviour of *Apis mellifera* in a Bee Flight Room

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Colonies of honeybees (*Apis mell.*) do not develop in a closed environment as good as in nature. In a bee flight room (BFR) colonies do rear brood, but they do not reach the swarming state. It was suggested (van Praagh, 1974), that not the amount of food, but the way food reaches the colony is a factor limiting colony development in flight rooms. The amount of food (carbohydrates and proteins) inside the colony does not correlate with the actual brood rearing activity of the colony. Younger larvae disappear by cannibalism (Czoppelt, 1980); leading to more than 60% uncapped brood. Cannibalism can be caused by a lack of protein (Woyke 1977, Weiß 1984). Apparently the colony misinterpretes the actual protein-situation. A high air humidity in a BFR leads to less than 50% uncapped brood at any time (van Praagh 1982)

Material, Methods and Definitions

Colonies of *Apis mell.* were kept in a BFR with controlled environment. The lighting conditions were as described by van Praagh, 1974. The temperature could be kept constant within 1 degree Celsius. Day temperature set at 23° C; night temperature at 19° C. The relative air humidity (rH) ranged +/- 5% of the presetted value. Absolute night humidity was set at 12g/kg.

Pollen collecting activity was studied under different rH values at daytime. 6g/kg, 12g/kg and 15g/kg water was used, resulting in a rH at 23° C. of 35%, 65% and 85%.

Grounded pollenbaskets were given in flat dishes. The size of the pollen particles ranged from sigle grains till 500 µm.

Every 40 min. the amount of collected pollen was measured by weighing the dishes and correcting for hygroscopy of the pollen.

The number of bees foraging at the dishes and hovering above them was recorded every five minutes by simultaneously photographing with two cameras in perpendicular directions.

The "collecting capacity" for each 40 minutes period, or for the whole run, was calculated as the surface underneath the "activity curve". The activity curve is the fitted curve through the number of collecting bees, counted every five minutes, plotted against time.

The amount of collected pollen in relation to the collecting capacity gives the "collecting efficiency" for the period under consideration. So this parameter is independent of the total number of bees collecting at that time.

Results

Comparing the activity curves of the population collecting by high (85% rH) and low (65% rH) air humidity the activity decreases when approximately 50% of the amount of pollen available is collected in dry air. By high humidity the population of gathering bees remains nearly stable. Not only number of bees gathering pollen decreases at low humidity, but also the collecting efficiency of each bee. Fig.1.

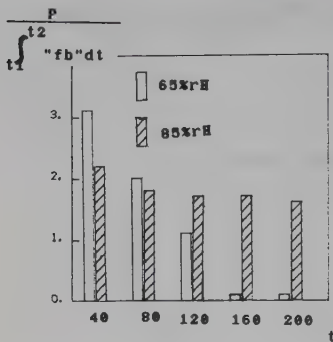


Fig.1 "Stable collecting efficiency at high air humidity"

p=amount of pollen collected in 40 minutes intervals

"fb"=number of foraging bees

"40" : t1=0 minutes - t2=40 minutes

"80" : t1=40 minutes - t2= 80 minutes

Mean values from 2x 25 runs. each time 60 gram pollen was offered

Adding grounded pollen at certain intervals keeps the collecting efficiency high. The same result is obtained, when such an amount of pollen is offered that it takes several hours to get the "easy collectable 50%" collected.

When the air humidity is raised from 65% rH to 85% rH at that moment when there is hardly any collecting activity anymore, then the collecting activity and the efficiency increases.

A high collecting efficiency is always correlated with a larger proportion of the collecting population hovering above the dishes. Bees stop collecting fine grounded pollen-grains (smaller than 100 μ m) after a while at high humidity.

Our data suggest that in both situations the finely grounded part of the pollen offered is collected first.

A larger part of the coarse-grained pollen can be collected by the bees at high humidity. In this situation more time is spent in the dishes, bees chewing the remaining particles. Apparently the bees can not gather efficiently from these particles at lower humidity. These grains do have to absorb water to become collectable. The absorbed amount of water gives those grains a gloss, but the grains do not "melt". Some charges of pollen do absorb so much water at higher humidity, that a smear of pollen develops. This smear on the other hand, can not be collected; it has a very low collectability.

The steady collecting efficiency for hours leads to a continuous flow of protein into the colonies. This is the more natural situation. Balancing the pollenflow into the colonies in time leads to a more natural storage behaviour of the protein-flow, resulting in a more normal broodpattern.

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Anticipation of Feeding Time in Honeybees

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Honeybees visit a food site where sugar water is presented every 24 hours at the correct feeding time (Beling, 1929). This ability is maintained under constant conditions (Renner, 1957) when no external time cues are present. In time training experiments in constant flight rooms as well as in the field it can be observed that bees usually arrive at the food site shortly before the actual feeding time.

Here I tested whether this anticipation of feeding time can be regarded as a phase-angle difference between an entrained circadian system and an entraining food signal.

MATERIAL AND METHODS

Honeybees of the South African race *Apis mellifera capensis* Esch. were kept in small hives containing about 1000 to 3000 bees, a queen and brood.

For the test the colonies were transferred to a flight room with constant conditions of light (LL), temperature ($25^{\circ} \pm 1^{\circ}$), and rel. humidity ($65\% \pm 3\%$). The activity of the bees was registered at the hive entrance by a photocell system and was recorded on an actograph (Miniscript Z).

In the first week of the experiments food was presented *ad libitum*. During the following one or two weeks food was available outside the hive for two hours per cycle only. For the remaining hours of the cycle the feeding dish was replaced by a similar empty dish. Inter-feeding intervals (T) of 22, 23, 24, and 25 hours were performed. The experiments ended with at least 5 or 7 days of *ad libitum* feeding (for details of procedures see: Frisch and Aschoff, 1986).

RESULTS AND DISCUSSION

Under constant conditions honeybee colonies showed freerunning activity rhythms. The activity pattern was characterized by times of common activity and rest of the members of the colony. In most cases periods were slightly shorter than 24 hours.

When the bees were periodically fed they quickly adjusted their activity to the feeding cycle. Synchronisation occurred with some advance or delay transients. In all cases (T = 22, 23, 24, 25 h) the peak activity appeared during the time food was available.

After some days of time training anticipatory activity occurred in the 23, 24, and 25 hour schedule. Duration of anticipation was positively correlated to the period of the feeding cycle: 3 hours in the 23, 4 hours in the 24 and 7 hours in the 25 hour time training. No activity in anticipation of the feeding time was to be seen in the 22

hour schedule.

After termination of the restricted feeding cycles activity was freerunning again. The initial phases of the freerunning rhythm corresponded to the preceding phases during restricted feeding.

Experimental data obtained from 19 colonies are summarized in Fig.1. Activity records during the steady state of entrainment have been normalized by expressing the data as percentage of the mean value of the inter-feeding interval, T . When activity exceeds the mean value this is regarded as activity time.

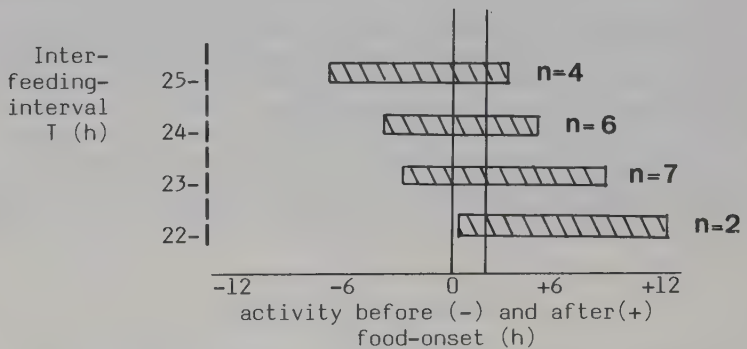


Fig.1: Activity time of Cape honeybee colonies under constant conditions of illumination, temperature and relative humidity. Colonies were exposed to feeding cycles with various inter-feeding intervals. Dashed bars represent the time span during which activity is above its mean level. Mean values of the colonies tested with the same inter-feeding interval are given. Time of food availability is indicated by two vertical lines.

The distribution of the activity pattern around feeding time changes systematically. Activity precedes feeding time in the 25 hour cycles, is equally distributed around feeding time in the 24 hour cycles, and lags behind feeding time in cycles shorter than 24 hours. Altogether the pattern of the bees' activity rhythm gives the impression of an entrained circadian system, which changes its phase-relation to a zeitgeber according to the period of the zeitgeber. In this view anticipatory activity represents the phase-angle difference between the periodical onset of food presentation and an entrained endogenous oscillation.

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Maintaining and Overwintering of Stingless Bee Colonies in a Flight Room

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Colonies of the Brazilian stingless bee, Scaptotrigona postica depilis, have been kept at our laboratories in Münster and Tübingen, Germany, for over 12 years. This Trigonine species is widely distributed in regions with tropical rain forests. The nest is found in tree cavities and has a protruding entrance funnel. During the European summer the colonies are hived in temperature-controlled observation boxes and are allowed to fly freely. At ambient temperatures over 20°C the bees forage and carry out garbage. Normally ample pollen is collected, but not enough nectar, so syrup has to be fed inside the nest. Overwintering is possible if the boxes are placed indoors and the entrance is closed. Then the colonies have to be supplied with all the food and cleaned at regular intervals. Alternatively the colonies can be overwintered in a flight room.

TECHNICAL DATA

The flight₃ room installed at our institute in Tübingen measures 6.0 x 3.4 x 2.4 m³. The walls and the ceiling are covered with creased aluminium foil. Bright indirect illumination is provided by 6 lamps each equipped with 3 tubes 65-80 W, operating in 3 phases. The tubes of type Philips TLA are providing a high proportion of UV light. A 13 hrs day/10 hrs night regime with 2 x 0.5 hrs dawn periods gives long day conditions. The daytime temperature is about 25°C and 22°C during the night. A high daytime humidity of 90% is catered₃ for by an evaporation system. Within the conditioned chamber a 14 m³ flight cage covered with a small-mashed nylon gaze is set up (Fig 1).

RESULTS

Up to six colonies of Scaptotrigona postica depilis were kept there, sometimes together with a nest box containing another stingless bee, Tetragonisca angustula (= Trigona jaty), and a number of honey bee colonies in magazine boxes or Kirchhainer nuclei. To facilitate entrance finding, 6 V bulbs were placed close to the nest and illuminated uptill half an hour after switch off of the main lights in the evening. In contrast to honey bees all the stingless bees regularly spend the night within the nest. Feeding stations with syrup, honey water, pure water and additional fluorescent dishes provided twice daily with grinded pollen were offered.

Nest boxes placed in the flight cage were within a day furnished by the bees with long entrance funnels typical for the genus Scaptotrigona. Guards controlled the entrance. Foragers were observed carrying out garbage. Some winters the stingless bees collected plenty of syrup, especially if odored with floral scent. In other cases the Scaptotrigona foragers detected the honey stores in Apis colonies and permanently robbed them, without visiting the feeding stations. In contrast to honey bees, pollen was only sparsely collected and carried to the nest. Therefore, the colonies had to be given pollen paste in order to stimulate and facilitate brood rearing. If this happened, then the brood produced was plentiful throughout the European winter, including drones and gynes. Once a young queen was

observed sitting on the box of an orphan colony. We put this queen into the queenless nest, but egg laying was later not recorded. Apparently the queen was either not accepted or not mated. Also initial steps in founding a filial nest occurred. At first an entrance funnel was built, then 4 honey pots were constructed in the cavity and in part filled, and some pollen crumbs were scattered about. As this happened in April/May, shortly before the colonies were moved out, there unfortunately was no opportunity to eventually observe a swarming process and the final events of a new colony separation.

There was no intraspecific competition observed at the feeding stations between foragers from the different Scaptotrigona colonies. Sometimes short attacks were seen on honey bees sucking syrup at the feeder. The Apis workers seemingly avoided any fight. Surprisingly they also did not defend their colony entrances and honey combs despite the robbing stingless bees. Interspecific food resource competition was detected between foragers of Scaptotrigona and Tetragonisca. The latter and much smaller bees were very aggressive toward Scaptotrigona foragers and bit them in the legs and wings. The stronger Scaptotrigona workers with their mandibels decapitated the little Tetragonisca bees, but failed to remove the seizing dwarf heads. We sometimes found a Scaptotrigona forager with more than 5 Tetragonisca capits attached and hence immobilized.

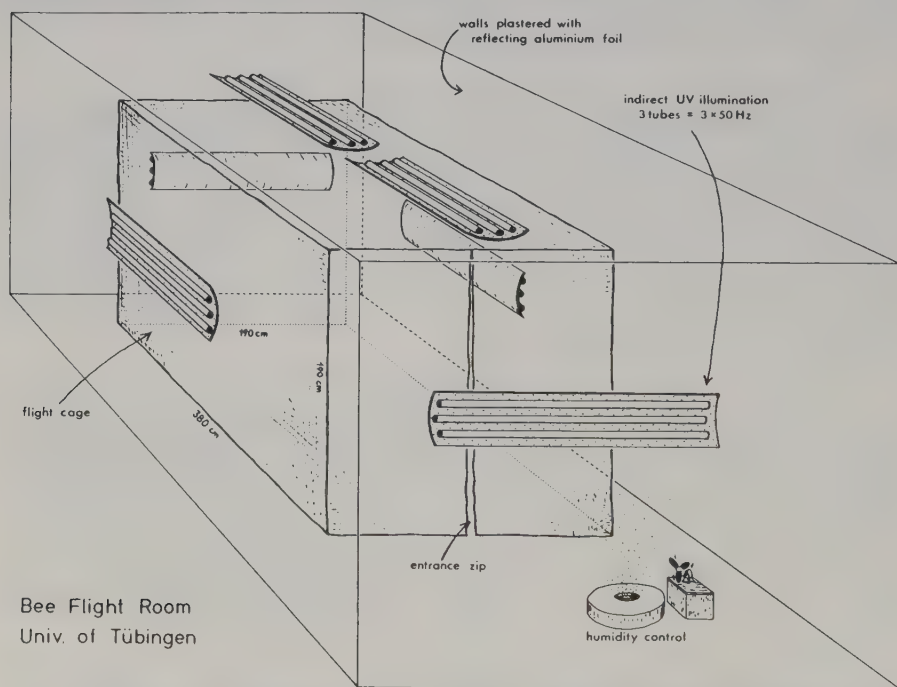


Fig. 1: Design and equipment of our flight room with the bee flight cage.

DISCUSSION

In summary it can be stated that stingless bee colonies can be kept for months in a flight room in a good condition. In contrast to honey bee colonies which presumably perceive flight room conditions like an autumn season, and consequently always reduce brood production and never rear queens and drones, stingless bees persist in continuing normal colonial life including reproduction.

FREE COMMUNICATIONS

Early Social Experience Affects Worker and Cocoon Discrimination in a Slave Ant Species

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Good evidence of imprinting-like phenomena has been recently presented in several forms of ants' social behaviour. The possibility that early social environment can account also for the origin and behaviour of slave-workers in natural dulotic ant societies has been here considered. In fact, the interspecific altruistic behaviour shown by the slave species towards its parasite is not explainable by means of the genetic sociobiological concepts of kin selection and inclusive fitness.

Since in nature the slaves accept their slave-maker as nestmate and care for its brood, we attempted to determine whether early olfactory learning is involved in these phenomena, studying both intra- and interspecific relationships as well as cocoon nursing behaviour of *Formica cunicularia*, a common slave of the slave-keeping *F. sanguinea*.

Preliminary results on ants paired in a laboratory aggression test indicate that *F. cunicularia* ant-workers, taken in the field from a pure colony, quickly discriminate between nestmates and *F. sanguinea*. Moreover they are able to recognize their own cocoons from the heterospecific ones (*F. sanguinea*, *F. lugubris*) which are rapidly destroyed. These results show that the slave condition in this species is not genetically programmed. On the contrary, social environment at the emergence of *F. cunicularia* workers strongly influences, when they become adults, both their cocoon recognition and care as well as their adult discrimination. In fact, workers supplied during a training period (the first fortnight of life) with *F. sanguinea* cocoons tend to recognize and destroy the homospecific one. Moreover, workers hatched in the presence of *F. sanguinea* adults behave towards this adoptive species as nestmates, whereas perform overt aggression against their natural sisters. This behaviour is quite different from that displayed by both control and socially deprived ants. In fact, the control group workers, supplied during the training period with homospecific cocoons, adopt and care only for them; moreover, the workers of this group housed with adult natural nestmates from hatching, accept these familiar ants and fiercely fight against aliens. On the contrary, ants grown up in absence of cocoons or adults show an evident deficit in the ability to recognize both brood and workers.

In conclusion, the social experience soon after the emergence of *F. cunicularia* ant-workers can well justify their enslavement in nature, generally explaining the interspecific altruistic behaviour of the slave ants, since both worker and cocoon discrimination in these host species appears to be much more based on fellowship (or conviviality) than on kinship.

Territorial and Patrolling Males of three European *Polistes* wasps (Hymenoptera, Vespidae)

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Males of three sympatric species of Polistine wasps (*Polistes nimpha*, *P. foederatus*, *P. gallicus*) were investigated in the surroundings of Florence during several summers, from the end of July to the beginning of November. In the middle and sunny hours of the day all these males gathered at conspicuous sunlit landmarks (poles, trees, bushes, buildings, etc.). They exhibited different mate-location strategies: *P. nimpha* a lek system, *P. gallicus* a mixed strategy with territorial males and wandering intruders, *P. foederatus* swarm patrolling.

Males of *P. nimpha* showed a territorial behaviour, patrolling and defending well-defined sunlit areas of varying size on hedges and bushes. A typical circular abdomen-dragging movement on particular leaves was probably related to the release of sex pheromones. Aggressiveness between neighbours included threat postures, pursuit flights, attacks and grapples falling to the ground.

A mark-recapture study in a population of *P. gallicus* indicated that 20-25% of the males returned to the same perches over a mean period of 14 days. If a wasp was seen in the same site on 3 or more census days, it was considered to be a resident male (R); if not, it was classified as a transient male (T). R males, significantly larger than T males, exhibited a territorial behaviour, while T males employed a wide-ranging patrol flight during which they made no effort to defend any exclusive territory. Females were rarely seen on perches and only a few copulations were observed, all by R males. Additional experiments, conducted in captivity in order to optimize the probability of male-female interactions, confirmed the success of R males.

In September, as the *P. gallicus* male population declined, *P. foederatus* males were found patrolling the same perches, becoming exceptionally numerous in October. A mark-recapture study revealed that some males (10-40%), although non-territorial, exhibited an attachment to a home-range, returning to the same area over a period of several days. These seldom or non-aggressive males followed roughly circular routes in swarms, stopping at common resting sites. Few copulations (sometimes with numerous males on one landed female and sequential mounts) were seen, all by long-term patrollers (captured in the same area on 3 or more census days). Immobilized females placed at resting stations elicited more responses from long-term patrollers than from short-term patrollers captured once, twice or unmarked.

The Effects of Honey Bee (*Apis mellifera*) Size on Behaviour

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Although worker honey bees do not vary greatly in size; there is 15-20% variation within a colony. It is well known that size is influenced by the size of the cell a bee is reared in. By altering the size of the cell imprint in wax foundation from which combs are built, bee size can be manipulated for experimental and commercial purposes. I quantified the effects of cell size on worker bee size, and of bee size on temporal polyethism, survival, and foraging.

Bees in 10 different colonies were allowed to construct combs from wax foundation of 5 different sizes (approx. 640, 700, 750, 800, and 900 cells/dm²). As bees emerged from these combs, they were caught, weighed, dried, weighed again, and various body parts measured; the volume of the cells they emerged from was determined as well.

Comb construction was normal for all cell sizes. Cell volume (ml) was proportional to cell area (mm²) (regression equation: $\text{vol} = 0.011 \text{ area} - 0.00418$, $p < 0.001$). Multiple correlations of bee size (fresh weight, dry weight, forewing length) with cell volume were usually significant, but not always, within bees of the same colony. Correlations with other parameters (head width, radial cell length, hind leg measurements, hamuli) were more variable and usually not significant. Brood production was less and the proportion of drones was substantially greater on combs with the largest "worker" cells.

Bees from the combs with the largest and the smallest cells of a colony were weighed upon emergence, individually marked, and introduced to observation colonies for behavioral studies. Because bees from large cells were not always the heaviest, cohorts of 100 large and 100 small bees were determined on the basis of weight at emergence (125 mg in 1983, 113.6 mg in 1984). Data on behavioral tasks were obtained by scan sampling between 17 Aug-3 Oct, 1983 (one colony) and 1 Jun-9 Jul, 1984 (two colonies).

There were no consistent differences in behavioral acts recorded for large and small bees within colonies. Moreover, the first day a given behavioral act was observed was not consistently earlier for either group of bees. Bee weight did not influence the day of first recorded flight or survivorship. In one colony (1984) large bees took foraging flights of longer duration; there were no differences in the other colonies. The time of year did not affect the results.

Kerr and Hebling (1964), *Evolution* 18: 267-270, working with Africanized bees, provided data which suggested that larger bees have accelerated temporal polyethism schedules. My results, and those of Nowogrodzki (*Bee Wld.* 65: 109-116) and Cideciyan/Waddington (pers. comm.), do not support that conclusion. Worker size may have little influence on worker bee behavior (Kerr and Hebling had marginally significant data). Alternatively, the behavior of Africanized worker bees may be influenced more by size than is true for European bees.

On Stimuli Releasing Male Courtship Display in *Blabera fusca* (Br.)

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In *Blabera fusca* (Br.), male courtship is triggered by contact with the tegument of a virgin female. This implicates either contact pheromone, or perception of the female by mechanoreception.

RELEASE OF MALE COURTSHIP DISPLAY AFTER ANTENNAL CONTACT

In all the observations, antennal fencing was the most efficient stimulus in releasing male courtship display, so mating was experimentally investigated with imaginal molt antennectomized males or females: virgin males paired with antennectomized virgin females spent more time in mating than with corresponding controls, little or no courtship was seen when antennectomized males were paired with virgin females: when contact was established, females became aggressive or tried to escape. Finally, antennae were implicated both in contact recognition and releasing information. Role of female antennae was enhanced in this experiment.

ANTENNAL STIMULUS CHARACTERS

It was demonstrated that courtship display was effective when male antennae were allowed to contact:

- * antennae from living females,
 - * female antennae carried by an isolated head in which brain was undamaged,
 - * numerous females antennae separated from several insects.
- The experiments shown that female antennae recognition by male is instantaneous. As there were no morphological differences between female and male antennae, the stimulus must not be purely mechanical and chemical(s) must be hypothesized. This pheromone is always present at antennal level; nevertheless, stimulus lability was demonstrated. Finally, data suggested the cerebral control by the female of stimulus-inducing male courtship. The fact that temperature modified courtship intensity was in behalf of volatility.

DISCUSSION: NATURE OF THE COURTSHIP-RELEASING STIMULUS

Courtship is released by chemical contact. Lability of the stimulus suggests evidence for volatile pheromone controlled by brain. As it was shown in *Blabera fusca* just after imaginal molt, two chemicals may be implicated in the stimulus:

- * a volatile pheromone necessary to induce search and sexual motivation,
 - * a contact pheromone less volatile instantaneously perceived by male, essentially at antennal level.
- Identical scheme may be hypothesized for more than two-days old females.

4.4 Role of Social Insects in Ecosystems

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The Fire Ant, *Solenopsis invicta*, as a Successful "Weed"

Walter R. Tschinkel

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Weeds are plants or animals adapted for the opportunistic exploitation of ecologically disturbed habitat. For this purpose, they often share several of the following biological characteristics. 1) Occurrence in disturbed, early succession habitat; 2) high reproductive rates and investment in propagules; 3) effective dispersal of propagules; 4) rapid colonization of available habitat; 5) rapid somatic (colony) growth; 6) early and continuous reproduction, before attainment of full somatic (colony) growth.

Many of the life history characteristics of the fire ant, *Solenopsis invicta*, can be interpreted as adaptations for a weedy life-style. Such an interpretation ties together a number of diverse research projects I have carried out over the last 5 years. To present a more complete interpretation, I have integrated the work of others with my own where necessary.

The fire ant is a mound-building myrmecine with very large colonies whose native home is in the Pantanal region of s. Brazil. It was accidentally introduced into the USA at Mobile, Alabama by unknown means, sometime between 1935 and 1940. Its rapid spread in the USA was largely through transport of nursery stock. Its present range is most of the southeastern USA which is warm and moist enough.

The rapid spread in the USA is in part the outcome of *S. invicta*'s nature. The primeval state of this region was unbroken broadleaf and pine forests, with rather scarce early succession communities limited to new sandbars, landslides, windfalls and clearings for Indian agriculture. By the early 20th century, most of the forest had been cleared for agriculture and living space, creating vast new opportunities for early succession species. Availability of so much disturbed habitat and a native ant fauna not fully capable of exploiting it set the stage for successful spread of the fire ant.

The first weedy property of *S. invicta* is thus its occurrence in disturbed habitat. Its abundance on lawns, pastures, roadsides and waste places is well documented. Less clear is its occurrence in relatively undisturbed native ecosystems. I surveyed two different pine forest ecotypes in the Apalachicola National Forest, south of Tallahassee, FL. The rolling pine uplands grow on deep sand hill of modest (10 m) relief. The water table lies 2 to 5 meters below the surface but is exposed in occasional ponds formed by the collapse of the underlying Karst limestone platform. In this ecotype, *S. invicta* is strictly limited to the margins of ponds and to grossly disturbed habitat such as maintained roadsides. A possibly native congener of generally similar habits, *S. geminata*, occurs throughout the forests.

In the second ecotype, the pine flatwoods, there is very little relief, the water table lies close to the surface and half of the area is shrubby wetland. In this ecotype, *S. geminata* is rare throughout, and even mild disturbance favors colonization by *S. invicta* which even occurs occasionally in undisturbed sites in the forest.

S. invicta's native home is a seasonally flooded riverplain, and the species has evolved special adaptations against flooding. Chief among these is the formation of floating mats of ants which can drift for weeks until the water recedes or they contact shore.

In addition to gross disturbance and flooding, specific disruption of the native ant community also favors *S. invicta*. Summerlin *et al.* (1977) and later Buren and Stimac (unpublished) showed that fire ants recolonized more quickly and increased their dominance over native ants after an area was treated with poison ant bait. They attributed this displacement of native species to the higher colonizing ability of *S. invicta*.

The second weedy property is high reproductive rate. Fire ant colonies invest 30 to 45% of their standing biomass in sexuals. A mature colony of 100,000 to 150,000 workers produces about 4500 sexuals per year (Morrill, 1974). High colony density often brings about mating flights with enormous numbers of sexuals.

The third weedy property is effective dispersal of sexuals in high altitude (over 100 m) mating flights. Queens mate in a matter of minutes and then disperse $\frac{1}{4}$ to 1 km or more (Markin *et al.*, 1971).

The 4th weedy property is efficient colonization of disturbed habitat. Newly-mated queens make the primary choice of habitat for colony-founding while still in the air. Once they land, they quickly dealate and proceed on foot, and rarely dig the founding chamber more than 4 or 5 meters from the point of landing. Newly-mated queens seem to prefer partly vegetated sites, landing with lower frequency on completely bare or heavily vegetated sites (though the latter is difficult to determine). Preferred sites are often colonized to densities of over 3 queens per m² in a single mating flight, and there may be 8 to 15 flights in a season.

Colony founding is strongly pleometrotic and competition among queens is apparently absent. The occurrence and degree of pleometrosis are driven by the local density of queens, on a scale of a few meters. This was demonstrated in an experiment in which the density of queens was manipulated, and the occurrence and level of pleometrosis, as well as the dispersion of nests in space and queens among nest were determined (Tschinkel and Howard, 1983). The results showed that the mean number of queens per chamber increased with queen density. At the same time, nests were ever more clumped within limited areas of the plots, and queens ever more clumped within a small fraction of the available nest chambers. Queens clump partly because they prefer pre-formed holes.

In several species of ants the advantage of joining a founding association rather than founding alone has been demonstrated to be improved survival and brood production (Bartz and Hölldobler, 1982; Pollock and Rissing, 1986). These advantages outweigh the risk of execution when workers reduce the nest to monogyny. For fire ants, there is optimal survival and brood production for groups of 4 to 7 queens. Larger and smaller groups have sharply reduced success (Tschinkel and Howard, 1983). The optimal nature of the relationship results from a mutual inhibition of brood production exerted by the queens, so that the per-queen contribution decreases rapidly with increasing queen number. Weight loss shows the inverse relationship. Oddly enough, in spite of the poor success of large groups, queens will join groups of any size, even hundreds. Perhaps the opportunity for selection against joining such large groups does not exist in *S. invicta*.

The improved brood production of pleometrotic foundation conveys a great advantage on the subsequent growth of these colonies. As long as colony growth is exponential (2 to 3 years) the relative size of colonies remains the same as it was after production of the first brood. A colony beginning with 50 minims will be 10 times as large as a colony beginning with 5 minims, throughout the exponential growth phase. This brings the importance of initial brood size into sharp focus, because the winner in the scramble for space will be the colony which achieves the largest size most rapidly.

This brings us to the 5th weedy property, rapid colony growth. The first means by which this is achieved is an extension of the principle of boosting the initial number of minim workers, and is accomplished by raiding nearby incipient nests for brood (intra-specific slavery). The winners of raids are usually those starting with the most minim workers, echoing the importance of pleometrotic founding. Workers from loser nests usually join the winners, abandoning their own mothers. Bartz and Hölldobler (1982) and Pollock and Rissing (1986) have described such brood raiding in the lab, but mine is the first field description. Incipient nests are linked by odor trails and brood is carried in both directions, but usually with net movement into one. Only minim workers take part, and raiding was observed only during the early incipient stage, before production of larger workers. Most raids are brief (less than 1 hr) and involved only 2 or 3 nests, but occasional raids were much larger (5 to 8 nests) and lasted for a day or two. One extremely large raid involved 80 nests over 35 days, took place over hundreds of meters of trails and finally resulted in the coalescence of minims and brood from all 80 nests into two. The boost to colony size resulting from such raiding is enormous. In essence, it is as though the surviving queen had begun colony founding with many-fold the reserves she actually had. The key to this success lies in the boost in initial numbers resulting from joining a foundress group, and this in turn makes success at brood-raiding highly likely, which in turn reduces the time it takes for colonies to grow to reproductive size.

It seems likely that much incipient colony "mortality" is the result of brood-raiding and colony coalescence. At the end of the claustral period, about 20% of naturally founded colonies are still alive and open the founding chambers. Within two months, only 1% are still alive, though in the lab, well over half the colonies still survive at this time.

The results of one of my recent laboratory studies can also be interpreted as an adaptation for rapid colony growth. It concerns the social control of the egg-laying rate of the queen and begins with the observation that ovarian hypertrophy, and therefore probably egg-laying rate, is positively related to colony size. Upon testing various colony members, I found that workers in broodless nests did not stimulate oviposition. Larvae, on the other hand, stimulated oviposition in direct relation to the logarithm of their number. The effect leveled off in 4 days. Removal of larvae from colonies caused the egg-laying rate of the queen to decline almost to near zero in less than 48 hr. Maximum egg-laying rates are between 100 and 200 eggs/hr. Such a queen lays up to her own weight in eggs daily, and is 75% ovaries by weight.

Only larvae in the late 4th instar stimulate oviposition. Younger larvae and pupae do not. A hypothetical mechanism is based on the fact that 4th instar larvae are the only colony members able to

digest solid prey protein. All others feed only on liquids. Perhaps these larvae play a role in processing protein for egg-yolk. Rapid movement of dyed food material from large larvae to workers to the queen and into the eggs is consistent with this hypothesis.

Late 4th instar larvae are thus linked in a positive feedback loop with the queen, driving the queen's oviposition rate to its maximum as rapidly as possible, to the limits of the colony's food resources. Not surprisingly, colonies appear to be strongly food limited.

The 6th weedy property is early and continuing reproduction, and is related to rapid growth. Typically, colonies take 3 to 5 years to reach the mature size of 100,000 to 150,000, but colonies as small as 15,000 or about 10% of mature size can produce some sexuals. About 50% of colonies produce some sexuals by the end of their first year, and 100% do so after two years (Markin et al, 1973). Presumably, there is some relation between the number of sexuals and the number of workers. Peak sexual production is in the spring, and they are released over 2 or 3 months in early summer. Mating flights are synchronized by heavy rains followed by warm days. A queen may produce 20,000 sexuals during her 5 to 7 years of life.

Several of the fire ant's biological properties cannot be classified as weedy. After completion of the brood-raiding phase, the colonies become strongly competitive and territorial. Queens can maintain colonies for 5 to 7 years, at which time they run out of sperm. In about 25% of colonies, they appear to be replaced with an inseminated but non-ovipositing queen already present and waiting in the colony. Colonies are thus potentially much longer lived than are queens, and a second avenue for reproductive success for newly-mated queens is acceptance into a mature colony and outliving the resident queen.

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SYMPOSIUM

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Radioactive Isotope Mark-Recapture Estimation of *Lasius flavus* F. Populations.

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To examine the role of any organism within an ecosystem it is necessary to know its density. With an ant species such as *Lasius flavus* it is possible to count the number of nests, but then, the number of ants in the individual nests, must be estimated. Counting the number of ants within a nest has always been a problem (Stradling 1970). It is reasonably simple, although time consuming and laborious, to dig up and count the workers, but it is destructive and precludes further work on the same nest. Mark-recapture is a technique which is not destructive, although with ants there are two main problems, firstly of obtaining a lasting, non-transferable mark, and secondly of collecting samples representative of the worker population. Various marking methods have been used but the best is that described by Stradling (1970), using the radioactive isotope phosphorus 32, fed to the ant sample in sugar solution, followed by a period of starvation so that the phosphorus is incorporated into body tissues and cannot be transmitted to unmarked ants. Stradling however did not conduct any field trials on *L. flavus* populations, and this is a less robust species than those he used. A series of experiments were set up to assess and refine the technique of Stradling with *L. flavus*, and it was subsequently tried on field populations of the ant, in several different areas of managed chalk grassland in Southern England.

METHODS

Two sets of laboratory experiments were undertaken, firstly to examine the details of attaining a suitable mark, and secondly to try mark-recapture using this technique on known populations.

The first set of experiments were to examine the following points.

- 1) The level of the mark achieved.
- 2) The necessity for a period of starvation prior to feeding the ants the isotope, so that the take up of the mark might be enhanced.
- 3) The necessary length of the starvation period after feeding so that there would be no contamination of non-marked ants.
- 4) The length of time for which the mark is distinguishable.
- 5) Whether any significant mortality is caused by the technique.

Duplicate sets from two nests, of groups of 100 worker ants in sealed petri dishes, each with a piece of moist sponge as a water source were prepared. A variety of treatments were then tested. Some sets were prestarved for one day, and some not, before being fed the phosphorus 32. A dilution of 1mCi of phosphorus 32 per 5 ml of 10% sucrose solution was used. This was given to the ants in small 'pots', the impregnated cotton wool of Stradling being found to be rather messy. Each set of 100 ants was given 0.1 ml of the solution, equivalent to 0.02 mCi. Controls were fed sugar solution only. After feeding the isotope, for 1 day, some sets were then starved for 3 to 5 days before being mixed with equal numbers of non-marked ants, and others were kept to be examined for the

level of the mark obtained. This was assessed in counts per minute using a hand held Geiger-Muller probe. In the mixed groups ants were assessed for contamination for up to 5 days after the initial mixing.

The best technique as determined by the above was used in the second set of tests where estimates were made of populations of up to 1,300 ants. This technique was then used in a set of field trials. Worker populations of 5 nests in each of 7 areas were estimated on two separate occasions. Samples were collected and returned beneath previously positioned stone slates (25 x 25 cm. and 2.5 cm. thick), under which the ants excavate numerous galleries, and congregate in warm conditions.

RESULTS

From the first set of tests the following details were determined.

1) Individual ants had marks after feeding of well over 100 c.p.m., enough to make them instantly detectable from the background levels.

2) Prestarvation of the ants for a day prior to feeding the isotope enhanced the mark in some groups, and this was subsequently done with all future groups of ants.

3) When the marked ants were mixed with non-marked ants there was little contamination. After a 3 day starvation period some slight contamination was observed after 5 days; up to about 30 c.p.m. above background levels. With a 5 day period of starvation this had completely disappeared. In practice a 4 day period was used.

4) The decay rate of the mark was equivalent to that of the half life of phosphorus 32, about 14 days, and the mark was detectable long enough for the purposes of mark-recapture.

5) No significant mortality was caused by the technique.

In the tests of accuracy in the laboratory trials the technique performed well, satisfactorily estimating 5 different populations of up to 1,300 worker ants, using the simple Lincoln Index formula.

In the field trials it was possible to collect samples of up to 1,000 ants at a time from a single nest. These were treated as described above. Initially the marked ants were released and a second sample taken 2 days later to assess the number of marked ants, but this was later extended to 4 days in line with Odum and Pontin (1961), to allow greater mixing of the sample with the rest of the population.

In the first set of estimates, worker populations were typical of known L. flavus nest sizes, ranging from 2,000 to 51,000. In two of the quadrats, sets of direct digging estimates, (5 nests per quadrat), mirrored the mark-recapture estimates, the average population being higher in a lesser grazed quadrat. The limited number of nests and the large variation between them make statistical comparisons difficult.

A second set of estimates produced results inconsistent with the first set. It was not possible to obtain satisfactory samples from beneath the slates on this occasion because of cold weather conditions. With the best samples the correlation with the first set of estimates was better.

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***Pheidole* Diversity in the Humid Tropics: a Survey from Serra dos Carajas, Para, Brazil**

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Pheidole is one of the most prevalent ant genera in the world (Wilson, 1976). For the Neotropics, Kempf (1972) lists more than 400 names under *Pheidole*, though the few surveys seldom report more than 20 species from a given locality. This situation may be an artifact reflecting low collecting intensity or indicate that *Pheidole* species are characterized by restricted distributions. The purpose of this study is to provide informations on the spatial heterogeneity and a minimum estimate of the number of ground inhabiting species recruiting to sardine baits in a tropical forest area. Prevalence among ant genera indicates that *Pheidole* is not typical of a given vegetation. Its high diversity in all regions makes this genus a good model for comparisons among regions. *Pheidole* in general nests in the ground or in rotten logs or stumps, is omnivorous and recruits to food items, and is thus suitable to quantitative collection using baits.

MATERIAL AND METHODS

As part of a survey of the ant fauna of the Serra dos Carajas, *Pheidole* were collected quantitatively near the town of Serra Norte (06°00'S, 51°20'W) close to the eastern border of the Amazon domain. Collection sites included 7 types of primary humid tropical forests, defined in terms of geographical, topographical and vegetational features. A group of 20 sardine baits (more in some places), set approximately 10m apart, were left for one hour before collecting ants in separate 30% alcohol vials. Individuals from a total of 229 *Pheidole* registers from 212 baits were cleaned with acetone, mounted and identified. *Pheidole* frequency data were compared between sites using the similarity index of Morisita (1959, viz Wolda, 1981) and the total fauna size estimated from the pooled samples by means of the lognormal distribution (Pielou, 1975). A complete data table along with detailed descriptions of localities can be obtained from the authors upon request.

RESULTS

We estimate that 100 or more ecologically similar *Pheidole* species may coexist in the region studied, a diversified area of humid tropical forest of the New World (Fig. 1). The actual number of species recorded at each locality, percentages of common occurrences and similarities between sites (Table 1) suggest a high degree of spatial heterogeneity in this fauna, although ecologically similar sites are often similar faunistically. With increasing sampling effort, previously unrecorded species were being added, with no indication of a plateau (Fig. 2), at a rate of approximately 12 species with each doubling of sample size.

	G	M	P	A	C	T	I
G	<u>13</u>	21	15	21	8	14	13
M	70.7 ^a	<u>21</u>	24	22	16	20	19
P	93.1 ^a	69.6 ^a	<u>20</u>	17	13	24	13
A	52.2 ^a	77.4 ^a	84.1	<u>15</u>	12	13	17
C	64.3 ^a	104.7	51.2 ^a	46.3 ^a	<u>11</u>	10	10
T	84.4	80.2 ^a	104.3	86.8	49.9 ^a	<u>9</u>	11
I	116.5	58.5	85.9	59.9 ^a	55.9	126.4	<u>10</u>

Table 1. Number of forest-floor inhabiting species of *Pheidole* (diagonal at each locality. Upper Right: percentages of species in common between localities. Lower left: Morisita similarity indices between localities (* <.05, ^a <.05, - >5% n. s.).

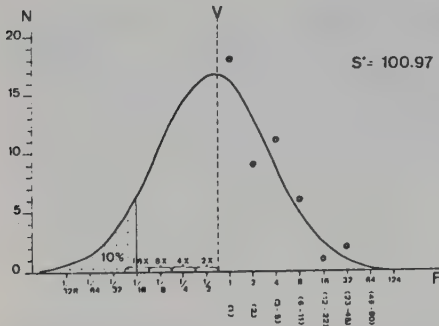


Fig. 1. Log-normal distribution for 212 registers of 47 *Pheidole* species collected at Serra dos Carajás, PA, Brasil (N= number of species, V= veil line, F= frequency class of number of registers, S*= estimated number of *Pheidole*). Data points do not depart significantly from the adjusted curve ($\chi^2 = 2.127$, $p > .1$).

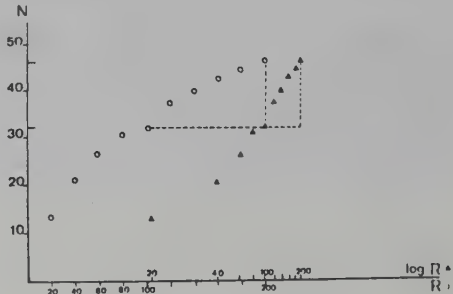


Fig. 2. Number of *Pheidole* species (N) registered (R) recruiting to Circa 200 sardine baits set at different primary forested habitats at Serra dos Carajás, PA, Brasil. Note double scale on X-axis.

DISCUSSION

Our data show that large numbers of *Pheidole* may occur sympatrically in humid tropical forests. Since only one type of sampling was used and since the distribution employed is probably conservative, we consider our estimate to be low. Several species sampled are clearly widespread dominants, although the majority were rare and 18 of the 47 found were registered at only a single sample site. Although faunal similarity between sites was often low, samples from more humid habitats (C, M, and A) and drier ones (G, P, T and I) tended to be more homogeneous, suggesting the existence of species distribution clines within the forest. Our results indicate that *Pheidole* may be a useful and relevant model taxon in studies of tropical biogeographical ecology.

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Ant Communities at Mt. Fuji, with Special Reference to Ecocline Between Scoria Grassland and Coniferous Forest

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Mt. Fuji is a dormant conical volcano, covered with thick scoria. The vegetation of this mountain is relatively simple and is a good example of xerarch succession. The climate is also relatively typical. These conditions seem to be good for analysing the factors which effect the biogeography of ants. The scoria grassland vegetation, such as Arabido-Polygonetum weyrichii alpini association and Cirsio-Campanuletum hondoensis association, is often associated with Myrmica kurokii, Formica lemni and Manica yessensis. Myrmica kurokii also inhabits Abies forest, but Formica lemni and Manica yessensis does not. Leptothorax acervorum is found in deciduous woodland and shrub. This phenological pattern was determined by a survey of the ecocline between scoria grassland and coniferous evergreen forest along a transverse path, called Ochudo (2310m-2400m), midway up the western slope of Mt. Fuji during August 18-20 and September 8-9, 1985.

METHOD

A set of traps of one-third diluted honey was distributed at intervals of five paces (about 3m) along the route. After 1 hour of exposure, ants in the traps were sorted and counted according to species. The data were then used to determine coverage and constancy. Coverage, defined as the extent of space covered by a species within a given area, was calculated by dividing the number of occupied traps by the total number of traps exposed at a given site. This method is similar to that

Table 1. Ant fauna and vegetations along a transverse path, Ochudo

Sampling sites	Coniferous evergreen forest									
	C ₄	C ₅	C ₁₀	C ₁₂	C ₁₈	C ₂₀	C ₂₂	C ₂₄	C ₁₇	C ₁₉
Whole ant coverage (ratio)	7 28	2 12	3 30	0 25	1 17	3 50	1 32	1 32	22 58	2 10
(index)	2	+	1	.	+	+	+	+	3	2
Coverage of each species (index)										
<i>Myrmica kurokii</i>	2	+	1	.	+	+	+	+	3	2
<i>Formica lemni</i>	+	.
<i>Leptothorax acervorum</i>
<i>Formica sanguinea</i>

Leptanilla japonica*: the First Bionomic Information on the Enigmatic Ant Subfamily *Leptanillinae

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The life of the ants of *Leptanillinae* has been an enigma in modern myrmecology for total lack of information on their ecology and behavior. Some of their known adult morphology, however, suggest that leptanillines have legionary habits as those of army ants (Wilson, 1971). Recently, I have collected a total of eleven colonies of *Leptanilla japonica* Baroni Urbani, in the broadleaved forest at Cape Manazuru, Central Japan. Colonies were obtained at all seasons except autumn so that the seasonal cycle of this species could be outlined. Further, some colonies were cultured and observed in laboratory plaster nests. Details of clarified new aspects of leptanilline biology are to be published elsewhere. In this paper, I first briefly review the life history of *L. japonica*, and second suggest an adaptive relation between cyclic brood production and an aberrant form of queen's feeding termed larval hemolymph feeding (LHF). This behavior has been also found in *Amblyopone* and *Proceratium* (Masuko, 1986).

Bionomics of *Leptanilla japonica*

Leptanilla japonica is a totally subterranean species. Colonies are monogynous and worker population is small, usually about 100 workers per colony. In addition to the adults, there are 100-200 immature individuals in a colony. Their development is strictly synchronized: Winter or spring colonies contain only the same-sized final-instar larvae. On the other hand, the brood of most summer colonies consist of pupae and eggs or minute larvae just hatched. My laboratory observation in *L. japonica* and field collection of captured prey from a colony belonging to a Formosan undescribed species (Terayama, unpubl.) collectively suggest that *Leptanilla* species are specialist predators of geophilomorph centipedes. In laboratory nests, trunk trails are extended all over the plaster floor. Following these trails, workers forage away from the brood pile. Although laboratory colonies with many growing larvae consume prey centipedes one after another, when once the larvae mature, prey consumption in the colony completely stops.

When the nest is disturbed, workers at once start transporting larvae along the trails. At this time, the workers do not use their mandibles to hold larvae. Instead, they, with the lower mouthparts, grip the peculiar appendages projecting from the larval prothorax. These appendages are obviously a specialized coupling device for transportation. Larvae have another strange structure unique to *Leptanilla*. It is located on each side of the 3rd abdominal segment and serves as an exudatory organ, i.e., gives hemolymph as a nutrient to adults, especially queens. Although this organ has been thought to be a single pair of spiracles that *Leptanilla* larvae have (e.g., Wheeler and Wheeler, 1976), I have found other, true spiracles with a SEM. The queen's nutrition is exclusively dependent on larval hemolymph. Even when feeding on captured prey is possible, the queen ignores it

repeating LHF.

About the time when larvae attain full growth, the frequency of queen's LHF is abruptly elevated. This change means the beginning of oviposition phase in Leptanilla japonica colonies. Most larvae in the colony are subjected to her activated feeding. Owing to this nutrition, she soon becomes physogastric. LHF has, however, no serious effect on maturation of larvae, which succeed in pupation, and the queen finally lays eggs in a brief burst.

The relationship among brood development, queen's oviposition, and predatory activity in Leptanilla japonica is summarized as follows: In the growing phase of larvae, intensive hunting of centipedes is conducted. The larvae consume the victims and grow quickly. During this period, the queen's gaster remain constricted. When the larvae mature, colonies go into the oviposition phase. The queen performs LHF actively and becomes physogastric. Prey consumption ceases and larvae pupate all at once while eggs are laid in mass. Thus another growing phase is set about.

LHF and cyclic brood production

I point out an important contribution of LHF to cyclic brood production. Leptanilla queens do not produce eggs until older larvae have matured. The queen, using LHF, extracts some energy from the mature larvae and converts it into production of new eggs. LHF is an easy and secure, thus adaptive way for queens to keep the synchronization between their mass oviposition and the brood maturation. The similar adaptiveness is expected in genuine army ants in the tropics. Little has been known about the queen's nutrition in such legionary ants (e.g., Rettenmeyer, 1963, Schneirla, 1971, Gotwald, 1982). At least in laboratory, army ant queens have never been observed to feed directly on prey animals, nor to be regurgitated food from workers. How do they obtain their nutrients needed for so large-scale egg production? LHF or LHF-like feeding utilizing (mature) larvae may be involved also in this case. As concerns this, it is to be noted that Rettenmeyer (1963) shortly described what appears to be LHF in workers of Eciton hamatum cultured in laboratory. Also in Leptanilla japonica, workers generally perform LHF. The possibility of LHF in army ants deserves closer attention.

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Colony Limits in *Solenopsis invicta* Buren

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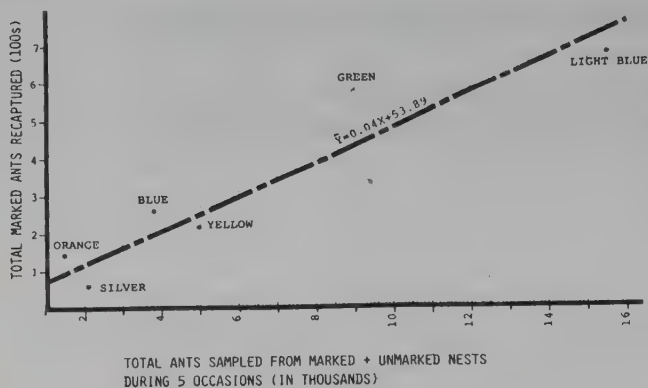
Sympatric single and multiple queen (mono- and polygynous) populations of *Solenopsis invicta* Buren occur in eastern and southcentral U.S. (Fletcher, et al. 1980, Greenberg, et al. 1985). Studies on the mosaic of mono- and polygynous colonies in a pasture habitat demonstrated their niche-specific foraging patterns (Bhatkar, elsewhere in this volume). Attempt was made to determine the limits of these co-occurring colonies.

MATERIALS AND METHODS

The mound nests in 0.2 ha circular plots were mapped using a surveyor's plane table. Head widths of the sampled ants were used to characterize the nests as mono- or polygyne (Greenberg et al. 1985). About 5-10% of 100,000-200,000 ants per nest were externally marked using distinctive cuticular spray paints by lightly stamping around the mound but not disturbing the suprastructure. Sequential sampling was done by thrusting a Fluon-rimmed 6 x 2.5 cm (20 ml) vial into the crust of each mound in 0.1 ha central area encompassing the marked mounds and capturing ants for 10 min. Cat food pellets (25-100 mg fish protein and lipid) were placed in the vials and distributed in concentric circles to observe the foraging boundaries of the colonies.

RESULTS

The mound density in mono- and polygynous areas varied from 150-200 and 500-1000/ha, respectively. The dispersion pattern of mounds in 4 quarters approached from random to regular ($R > 1$, Clark and Evans 1954) from spring to winter. Regular spatial distribution of nests was regarded as an outcome of overlap in food utilization (Levings and Tranter 1981). No mixing of distinctively marked ants occurred in the samples collected from monogyne nests, thus each nest acting as a monodomous, monogynous colony. A linear correlation was observed between the marked and unmarked ants from each mound during the 5 d sampling periods. There was a mixing of marked workers from the marked to the surrounding mounds in the polygyne area. The sampled workers from all the mounds in 0.1 ha area showed a marked linear correlation, indicating a steady-state worker traffic among the nests at any given time as a polydomous supercolony. Such colonies may stretch over hectares of area, limited only by the patchy distribution of monodomous, monogynous



STEADY-STATE TRAFFIC OF MARKED WORKERS IN 0.1 HECTARE
POLYDOMOUS *SOLENOPSIS INVICTA* PLOT, A&M FARM (JAN 1986)

colonies in a pasture-type habitat. The marked foragers from several nests foraged at the same food stations in a polydomous colony area, while foragers from marked monodamous colonies monopolized the adjacent food stations to their nests, thus keeping separate foraging boundaries amongst them. In addition to spatial, they fluctuated temporally, different colonies foraging actively at different times.

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Seed Predation by Harvester Ants in the Pinacate Desert, Mexico

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In desert areas, seeds of annual plants function as resistant life-history stages of these plants during long periods of low soil moisture. These seeds are also the primary food resource of a group of postdispersal predators: ants, rodents and birds. Brown and Davidson (1977) have argued that these predators are often resource limited and that they can constitute a strong pressure upon many ephemeral plant populations. At the end of short growth pulses, seed consumers can remove a considerable fraction of the potential seed reserves in the soil. Then the density, species composition and relative abundance of annuals could be affected by means of this activity.

The role of ants as vectors of seed dispersal is significant in plants of many taxonomic groups. A preliminary survey of the literature, given by Nesom (1981), reveals that at least 223 genera in 63 families include known myrmecochorous taxa. Davidson and Morton (1981) have shown that dispersal of diaspores is directed toward favorable microhabitats where nutrients are concentrated, and mention that myrmecochory can have a pronounced effect on seed plant dispersion in the Australian arid zone. The aim of this work is to examine the role of granivorous ants in a desert habitat from the viewpoint of their interactions with the annual plant community and finally to lead into the subject of seed predation and seed dispersion.

MATERIAL AND METHODS

The study was conducted in the Pinacate desert area which is located in the Northwestern coastal plain of the Gulf of California, Sonora state, comprising the Sonoita river and the Altar desert. The mean annual precipitation is approximately 60.7 mm, but wide and erratic fluctuations occur. The natural vegetation is a microphyllous desert vegetation with *Larrea tridentata*, *Ambrosia dumosa* and *A. deltoidea* as characteristic species. A general description of the climate, geomorphology, soils, vegetation and fauna of the Pinacate desert area was given by May (1973). We worked with three species of ants: *Pogonomyrmex* sp., *Novomessor cockerelli* and *Veromessor pergandei* in three selected sites. Two squares (50x50m) were set up at each study site. Seed resource availability was estimated sampling the soils from late winter 1981 to spring 1986. Soil within a 100 cm² quadrat was collected to a depth of 1 cm from each of 25 (100 m²) quadrat plots where observations on the ant colonies were made. The composition of the diet of the ants was determined by removing and identifying seeds from returning workers, and data on the contents of nest granaries were also obtained. Estimates of seed production by the more common plant species were made. Estimates of plant density were made using random quadrats and these were multiplied by the number of seeds per plant.

RESULTS

Seed production in semi-arid regions is geared to erratic rainfall and typically occurs in large pulses with many plant species producing seed over the same period. In between pulses, the seed reserves available to ants gradually decline due to scattering, burial and germination. For this reason, the distribution of seeds in the soil was patchy and the variance in density among samples from any given month was highly skewed. Three species constituted 76 % of the total number of seeds extracted from the samples: *Dalea neo-mexicana* (60 %), *Schismus arabicus* (12 %) and *Plantago insularis* (4 %). Differences among seasons with respect to seed densities in the soil were highly significant, with densities being highest in winter, spring and early summer. Our records of plant phenologies and soil seed reserves in this area indicate that seed drop in the annuals *Plantago insularis*, *Chaenactis stevioides*, *Sphaeralcea coulteri*, *Dalea neo-mexicana* and *Schismus arabicus* occurs during the winter months. Most seed drops in the perennials *Larrea tridentata*, *Ambrosia dumosa* and *A. deltoidea* occur from spring to mid summer. These were the commonest species in the diets of the ants. There was remarkable consistency in diet among the colonies of each species in a given season. Very occasionally a colony deviated from the others by taking an unusually high proportion of one of the seed species. Presumably, the ant species did not partition the food resource according to the major food type. While arthropods and plant parts other than seeds were occasionally taken by *Novomessor cockerelli*, seeds made up 90-95 % of the items collected by foragers. The seasonal foraging activity of *Veromessor pergandei*, as estimated by its effective foragers, was correlated with the mean seasonal density of all seed species of the soil (15 species) and with the density of *Dalea neo-mexicana*, *Schismus arabicus* and *Plantago insularis* seeds in particular. There was no correlation between the seasonal activity of the other two ant species and seasonal seed densities. Seed size preferences of *V. pergandei* were generally consistent from season to season. Briese and Macauley (1981) have shown a qualitative correspondence between periods of seed production by several plant species and the proportion of those seed species in the forage of five species of seed-eating ants in Australia. Preliminary data from the Pinacate desert may suggest a similar pattern of seed consumption.

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Ant Communities around the Federsee and the Schmiechener See: a Comparative Study of two Nature Reserves in Southern Germany

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In continental Western Europe most of the land has been in agricultural use for centuries. Forest management was intensified about 200 years ago. Consequently, areas still carrying undisturbed arthropod communities which had not been altered by man-made habitat changes and insecticide application are very rare. The aim of this field study was to monitor ant communities in two nearly untouched study sites in Southern Germany.

METHODS AND STUDY SITES

Ant communities were studied since 1980 in and around two humid land nature reserves in Baden-Württemberg by pitfall traps and search of nests. The survey was organized as an intensive long-term field study in a number of representative sample squares. The two study sites are: 1. Federsee. Nature reserve for the past 75 years. A typical fen district of 50 km² in Upper Swabia with all types of bogs ranging from upland moor to lowland moor. On 64 test plots within and outside the nature reserve a total of 900 pitfall traps was placed. 2. Schmiechener See. Nature reserve of 5.1 km² for the past 13 years. A typical silted lake in the Jurassic chalk district, situated in the periphery of the Swabian Alb. The Schmiechener See differs from the Federsee by its greatly fluctuating water levels, resulting in a noticeably uniform sedge plant community. 260 pitfall traps were positioned on 20 test plots.

RESULTS

Rich ant communities were found in both study sites. In the Federsee area 28 species were recorded. Amongst the fen ants discovered there are the very rare species *Formica uralensis*, of which a remarkable relict population of 280 nests was registered, and furthermore *Sifolinia winterae* and *Harpagoxenus sublaevis*, both represented only by a few sampled specimen. In the Schmiechener See district merely 6 ant species were collected in the nature reserve itself, mainly common pioneers. 10 more species were found in the vicinity of lake borders. Altogether the following ant species can be listed: *Camponotus ligniperda*, *Formica rufa*, *F. polyctena*, *F. pratensis*, *F. uralensis*, *F. truncorum*, *Raptiformica sanguinea*, *Coptoformica exsecta*, *Serviformica fusca*, *S. picea*, *S. rufibarbis*, *Dendrolasius fuliginosus*, *Lasius niger*, *L. alienus*, *Chthonolasius flavus*, *Ch. umbratus*, *Ch. mixtus*, *Myrmica ruginodis*, *M. laevinodis*, *M. gallieni*, *M. scabrinodis*, *M. sabuleti*, *M. vandeli*, *M. schencki*, *M. lobicornis*, *Sifolinia winterae*, *Tetramorium caespitum*, *Leptothorax acervorum*, *L. muscorum*, *Harpagoxenus sublaevis*.

CONCLUSIONS

Both the Federsee and the Schmiechener See nature reserves are important humid habitats for populous ant communities in the mountain region north to the Alps, in the state of Baden-Württemberg. This comparative ant survey clearly indicates that the large Federsee reserve consisting of highly diverse biotopes is carrying a much richer myrmecofauna than the smaller Schmiechener See reserve. The latter could be improved by including more of the lake border areas, which today are used for agriculture.

Geographic Variability of Myrmecofauna of Urban Areas

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University of Helsinki, Finland

The main factors determining the expansiveness of ants in urban areas are the size and diversity of the geographical range of the species and the location of the town with respect to the boundary of its range. Urban myrmecofauna is dominated by species with large ranges (extending over several biomes), thus typically eurytopic forms. But they are highly expansive only in central parts of their ranges. The adaptability of these declines towards the peripheries.

This phenomenon has been analysed for the western Palaearctic along a geographical transect crossing three climatic zones: boreal-temperate-Mediterranean and the respective transect of plant formations: taiga (northern and southern)-mixed forests dominated by conifers, and deciduous forests-evergreen scrub.

In towns of the boreal climatic zone, where boreal and Eurosiberian species are dominant, myrmecofauna is poor in species and individuals, as the range of boreal species is small and that of Eurosiberian species is little diversified. In both cases these are oligotopic species (with small ecological amplitudes), mostly hygrophilous and thermophobe forest dwellers (these are unsuitable characters to live in urban habitats).

In the moderate climatic zone, urban areas are colonized mainly by Palaearctic species. This element is characterized by the largest and most diverse ranges, covering 5-6 biomes. Virtually all Palaearctic ants are able to colonize urban habitats, and their proportion by number often exceeds 90% in urban communities. The next position is occupied by the European element (50% of the species are able to cross the barrier of urban pressure, and they account for 25% of the number of ants in urban communities). The ranges of European species are rather small but relatively diversified.

Urban areas of the Mediterranean zone are colonized by about half of the species of each zoogeographical element. Their proportions are different. Mediterranean species are dominant, and also other species associated with this zone, such as Euromediterranean and Mauritanian, are abundant. Ants living in the moderate climate (Palaearctic, European) are poor in species and individuals.

These patterns can be modified by local environmental conditions.

Structure and Origin of Ant Communities of Warsaw

Bohdan Pisarski* and Wojciech Czechowski

Institute of Zoology, PAS Warsaw, Poland

Warsaw is located on the site of a potential linden-oak-hornbeam forest (*Tilio-Carpinetum*). Urban green is heterogeneous: it is made up of replacement lawn communities of the type of moist meadows (*Arrhenatherion*) or pastures (*Cynosurion*), with trees typical of deciduous forests. This is reflected in the composition of myrmecofauna (59% - open area species, 41% - forest species). It consists of 38 species (81% of the regional fauna), of which 35 occur in the suburbs, and 27 in the managed urban green of various types: 18 species in semi-natural wooded areas and 23 on lawns.

The mean density of ants on lawns is 700 individuals/m² (1030/m² in parks, 800/m² in streets, 270/m² in housing estates). Two species are dominant, *Lasius flavus* (66% on the average) and *L. niger* (27%). The absolutely constant species consist of *Myrmica rugulosa* and *L. niger*.

The species composition of ant communities from various types of urban green is similar. There are, however, differences in the community structure between habitats. Lawns in parks are inhabited by 7-14 species, the dominant species is always *L. flavus* (55-96%). Ant communities occurring on lawns in housing estates consist of 2-6 species, and they are always dominated by *L. niger* (57-79%). Ant communities along streets are of an intermediate character: they are made up of 3-7 species, and they are typically dominated by *L. flavus*, rarely by *L. niger* (52-92%).

When going from parks, through streets, to housing estates, the proportion of ants active on the ground surface (*L. niger*, *M. rugulosa*) is increasing in ant communities at the expense of ants living below the surface (*L. flavus*, *Diploporhoptum fugax*). This results from differential kind and intensity of gardening treatments in various types of urban green (mowing, turning over the soil, chemicals).

The zoogeographic composition of the myrmecofauna of Warsaw is predominated by Palaearctic and south-Eurosiberian species. Their proportions are increasing with urban pressure. Urban habitats enhance ants with large ecological amplitudes: eurytopic and polytopic. As *L. flavus*, *L. niger*, and *M. rugulosa* are the dominant species, the bulk of urban ant communities consists of Palaearctic, eurytopic (ubiquitous), and expansive forms.

FILM PRESENTATION:

Red Wood Ants - Hard Workers of the Forest**Helmut Barth and Daniel Cherix****Barth-Film, Baltingerstraße 14, 7951 Sulmingen, FRG****Museum of Zoology, Place de la Riponne 6, 1005 Lausanne, Switzerland**

The film producer (HB) and the scientific adviser (DC) followed two purposes in making this 45 minutes film. The first one was to show the entire life-cycle of the red wood ants (Formica rufa group), the second one to feature some recent discoveries as well as unknown behaviours found within these species.

It took three years to realize this film. Part of it was made in the swiss Jura, where the second author has been working for about ten years on a super-colony of Formica lugubris Zett. involving about 1'200 nests connected to one another. The other part, related to brood development, was filmed in Germany (Biberach/Riss) with Formica polycтена Foerster.

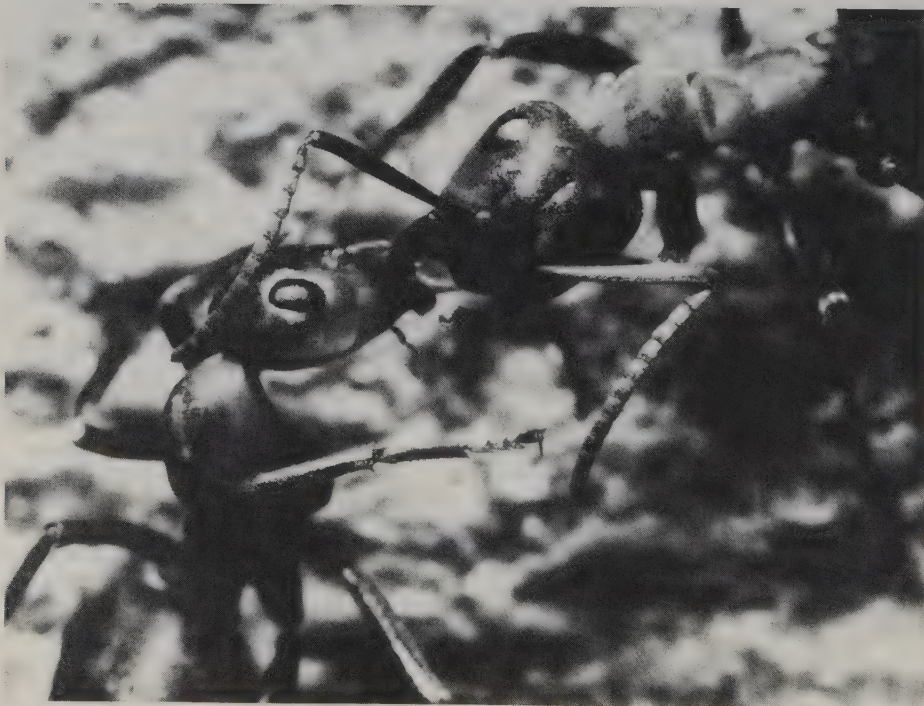


Photo 1. -- Close up view of liquid food exchange between two workers of *F. polycтена* (H. Barth).

To take macrophotographs you can use a flashlight, but to film red wood ants at very short distances puts you to a lot of troubles. These ants are very mobile and get immediately excited. It is even more difficult when filming inside the nest within brood chambers for example. To get rid of these technical difficulties requires different methods and techniques like the use of a special bellow with continuous sharpness. One of the methods was to open half of a nest and to work under a tent so that the "Innendiensttiere" were not influenced by natural light. Illumination was provided by glass fiber optic system. Moreover very sensitive colour film (28 DIN) allowed these extreme close up views, filmed at slow motion. In some scenes the subject was close to less than 3 cm from the camera lens (see photo 1). It was therefore possible to film one egg-laying queen, the carrying of eggs by workers, the feeding of larvae, the formation of worker cocoon as well as emergences of young alates females and workers. Sequences in which just freshly emerged individuals help each other to get out of their cocoon.

Another method was to build an unusual "Formicarium" consisting of a 3 cm wide nest ; one side covered with glass and black paper, the other side being fully illuminated with a lamp. To keep ants under such conditions required a very accurate adjustment of temperature and humidity otherwise queens will stop egg-laying.

Through careful observations it is possible to show for the first time the nuptial dance of alates females. The tending of aphids by foragers of *F. lugubris* (up to 6 times enlarged) as well as predation on the same tended aphid population are also shown.

The last part of the film is concerned with red wood ant protection in Germany.

ACKNOWLEDGEMENTS

This work was supported by the Institut für Film und Bild in Wissenschaft und Unterricht (FWU), Munich and the Bavarian Broadcast. Thanks are due to the Museum of zoology in Lausanne.

This film is available in two parts at the FWU, Institut für Film und Bild, Postfach 260, 8022 Grünwald :

- 1) Das Jahr der kleinen roten Waldameisen (20 minutes)
- 2) Brutbiologie der kleinen roten Waldameisen (13 minutes).

FILM PRESENTATION:

**Feeding Mechanisms of *Messor capitatus*,
a Harvesting Ant****B. Darchen***Station Biologique, 24620, Les Eyzies, France*

M. capitatus lives in the xerophilous and calcicole lawns of south-western France.

In the soil, it stores numerous seeds which will be eaten later.

This film shows how an insect, equipped with a licking organ, eats dry and tough food.

We compare the filtering organs of this ant and the ones of Camponotus herculeanus, a polyphagous ant.

Contrary to C. herculeanus, M. capitatus' stomach receives only liquid foods. M. capitatus has also the particularity to possess an extraoral digestion of seeds.

For more details, see References:

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The Adaptation of Termites

(*Nasutitermes* sp. - *Termitidae*, *Nasutitermitinae*)

to Amazonian Inundation Forests

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White water inundation forests ("várzea") are annually flooded by the waters of the Amazon which show level fluctuations of 10 m on the average (Junk 1984). Plants and animals are well adapted to these periodical changing conditions (Irmler 1981). Termites are important wood decomposers and their adaptation to changes in the supply of dead wood due to the inundations is focused in this paper.

MATERIAL AND METHODS

The study area is a dam-like place of about 300x80 m in the forest on Ilha de Marchantaria, an island in the upper Amazon (Rio Solimoes) near Manaus. A line was marked along the main axis of this "dam", the position of all termite nests in relation to this line measured, and all nests marked. Monthly, the presence or absence of termites was controlled in each nest, and the appearance of new nests was registered. The mortality rate was calculated from the percentage of the originally registered nests that had "died" (showed no sign of being inhabited by termites) during 9 months. - To determine altitude-dependent structure of the vegetation and dead-wood biomass (WB) an area of 5x60 m, subdivided into 5x5-m-plots (numbered 10 to 22), was marked vertically to the "main axis", covering all different altitude levels of the "dam". In each plot all trees of more than 3 cm ϕ were identified, the diameter measured and the height estimated. Then the whole dead-wood on the ground of each plot was sampled separately and weighed (from a subsample taken to the laboratory the fresh-to-dry-weight relationship was determined after drying 4 days at 100° C; all biomass data are given in d.w.). - On two additional plots of 5x15 m ("A&B"; situated at the height of plot 19) all litter was taken off, and the monthly wood litter fall was sampled over one year except during the inundation. In 1985 the high water margin was exactly in A&B and allowed to sample the dead wood deposited at the water border line.

RESULTS

1. The area divides into three different vegetation zones corresponding to the height of the ground and the related time of inundation. The lowest tree-covered zone ranges from 23 to 25 m above sea level (plots 10-16), with trees 4-7 m in height and consisting mainly of two species: *Crataeva benthamii* and *Vitex cymosa* (hereafter called C-zone). The dead-wood biomass is low: 3.58 t/ha. Between 25 and 26 m above sea level (plots 17-19) the diversity of the tree species is much higher; their height is about 10-15 m and the dead wood biomass amounts to 10.38 t/ha (= M-zone). Plots 20-22, above 26 m, are dominated by *Guarea trichiloides* with trees being 20-25 m high, but the biomass of the dead wood being only 5.86 t/ha (= G-zone).

On plots 17-19, a high amount of small branches (about 1-10 cm ϕ) is found lying packed in bands almost parallel to the water margin. This is the dead wood deposited by the flood at the high water line. It seems to be this material which makes the dead wood biomass in M so high. In 1985, the amount of deposited material (LF) was determined as 1.18 t/ha. This is 20 % of the annual litter fall from trees (LT; 5.98 t/ha).

With some caution a decomposition coefficient can be calculated for the M zone using $k = L/WB$ (with $L = LT + LF$). - The caution relates to the fact that the ecosystem should be in a steady state (Olson 1963) which might not be applicable to such an "open" system as the inundation forest). K is relatively high: 0.690, which means that 2/3 of the annual litterfall is decomposed in 1 year, and 95 % of it in 4.3 years.

2. Only the Nasutitermitinae are found to be important wood-feeding termites in this area, with 5 species identified up to now: Nasutitermes macrocephalus, N. surinamensis, N. cf. corniger, N. cf. ephratae and N. cf. tatarendae (identified by Dr. S. Bacchus, Brit. Mus. London). All species build their nests on trees; no epi- or endogeic forms occur due to the inundations. Of 90 originally recorded nests, 12 had "died", and 16 new ones appeared in the 9 months, which means that 17.7 % of all nests disappear in 1 year, whereas the renewal rate is 23.6 %/yr. Thus, the whole population is exchanged within 4.2-5.6 years. The mean density of nests is 86/ha, but their distribution within the vegetation zones reveals that the M zone with 150 nests/ha has twice the density of G (81 n./ha) and three times the density of C (47 n./ha).

3. The lower parts of the forest (C) not only produce less living and dead wood biomass due to the prolonged flooding, but they suffer an export of dead wood material which is accumulated at the high water margin. The distribution of the termites may in part be due to the structure of the vegetation (less places for safe nesting in the small trees of C), but this would not explain the weak nest density in G.

Although every year the high water level is not 26 m, seen over a period of years it is quite constant: Since 1980, in 4 out of 6 years the high water level was between 26 and 27 m. Thus, over several years an additional quantity of dead wood has been deposited in M by the flood. The fact that dead wood is the main resource for the termites, their mortality rate (4-6 years for total population exchange) as well as the decomposition coefficient (95% of annual input decomposed after 4.3 years) indicate that the density and distribution of termites is not accidental but rather the consequence of their flexible reaction to middle-term changes in the supply of dead wood due to the floodings. The zone (M) with the highest supply of dead wood is colonized predominantly and will consequently be most active in terms of decomposition.

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Contribution to the Study of Humification of Tropical Forest Soils: the Role of a Humivorous Species, *Thoracotermes macrothorax*

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This study is part of a broader research work dwelling on the role of termites (wood, fungus-comb and humus feeding) in the humification of tropical forest soils. The humus-feeding termite *Thoracotermes macrothorax* is active in the top-most 6 inches of the soil, over an area and within an approximately 12 feet radius from the nest. It coats its chamber and subterranean galleries with its own faeces, which invariably have a higher carbon content than the surrounding humus.

An electron-microscopic scanning of faeces (1), (2) and (3) emphasizes the building up of organo-mineral aggregates, consisting of spindle-shaped mineral particles which themselves are adsorbed on clearly recognizable vegetal debris, vegetal granules and on bacterial accumulations surrounded by polysaccharide secretions. A certain amount of these bonds persists after alkaline reagent extraction (pyrophosphate + soda), which emphasizes their stability. Thus, a certain amount of organic matter (humin) is included in the faeces, being locked by mineral particles.

MATERIALS AND METHODS

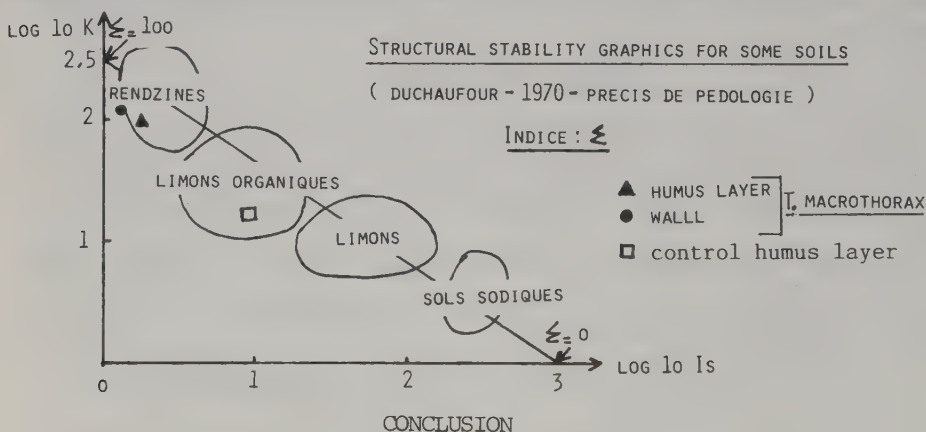
- Granulometric method: Soil particles can be classified according to their size range as follows: coarse sand 2.0-0.2 mm, fine sand 0.2-0.02 mm, silt 0.02-0.05, fine silt 0.05-0.002 mm and clay <0.002 mm.
- Exchangeable cations are extracted with 1N ammonium acetate at pH 7.0.
- Available phosphorous is measured colorimetrically by the molybdenum blue method.
- The quantity of total Fe is found by atomic absorption and total Al by emission in reduced plasma.
- Organic C is measured by carmograph, total N by Kjeldahl method.
- The nature of nitrogen compounds are determined by studying the products of acid hydrolysis.
- The humic acids are determined according to the method of Duchaufour with a mixture of sodium pyrophosphate at pH 9.7 and sodium hydroxide.
- The structural stability Is test, by the method of Henin.
- The permeability: K test, filtration velocity of water.

SUMMARY OF THE RESULTS

A pedological study of termite feeding ground as opposed to an untermited one presented the following evidence:

- a granulometric study reveals the animal's selectiveness for certain mineral elements according to their building properties, i.e. clay and coarse sand,
- an enrichment of exchangeable cations (Ca^{++} , Mg^{++} , K^+) in the wall and the humus under the influence of *T. macrothorax*,

- the high iron and aluminium contents emphasize the extent to which the insect accumulates those minerals from the ground,
- the termitted area contains twice as much phosphorous than the un-termitted one,
- the content of organic matter is always higher (9% vs. 4.2%),
- the distribution of humic compounds (fulvic acids and humic acids) and of humine (unextractable carbon) is particularly interesting: humus *T. macrothorax*: FA/HA = 1.09; wall: FA/HA = 0.88; control humus: FA/HA = 1.86. (Humic acids, which are nitrogen-rich macromolecules, display an interesting colloidal property by forming very stable compounds with clay. This binding process leads to a significant mineralization and accumulation of humic acids).
- the percentage of hydrolyzable nitrogen is higher in the humic layer occupied by termites than in the control layer (70%/82%), thus showing the stability of organic nitrogen compounds, which is probably due to the affinity of the nitrogenated fraction of humic compounds for some minerals;
- under the electron microscope, it appeared that inside the nest walls the bacterial microflora is practically insignificant. For this reason, we assume the addition, during nest building, of a bacteriostatic agent in order to suppress the traditional humus-degrading agents.
- both the permeability K test and the structural stability Is test point towards a better structural stability in the presence of *Thoracotermes macrothorax*. (The structural stability is the more important ecological factor, which shows the fertility of soils). (4)



By incorporating its faeces (which contain stable organomineral microaggregates) and sequestering some part of the organic matter, *Thoracotermes macrothorax* reinforces the structural stability of the soil throughout the lifetime of the termite mound.

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Distribution of Termite Mounds in a Drainage Basin of the Sudanian Zone (Côte d'Ivoire)

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This study is part of a multidisciplinary programme analysing the main driving variables involved in a model of water dynamics (runoff, infiltration rate, outflow) in a drainage basin of the sudanian savanna, North-East of Côte d'Ivoire (Lepage and Tano, 1986). Two main outcomes are expected from this work : to understand the factors (soil, vegetation) influencing termite distribution, and to identify the major constraints on cultivation (erosion patterns, soil water content) for a better management of the area.

THE STUDY AREA

The drainage basin, 136 ha in area, is located 8°28' N, 7°35' W. Average rainfall amounted to 1400 mm.yr⁻¹ (1384 mm in 1985, Chevallier *et al.*, 1986). Nearly 70% of this total is concentrated within three months, July to September. Five months (January to March, November-December) are virtually dry. This highly contrasted seasonality give rise to intense erosion activity.

Slopes varie from high gradient on the bottom land (10%) to gentle on the top (1-2%). Eight soil types have been identified (Fritsch *et al.* 1986), roughly distributed along with the topography : clay and sand hydromorphic, yellow and brown ferruginous, red and indurated ferrallitic, iron crusted and iron-pan soils.

Twelve vegetation types (or surface features and land use) have been recognized (Valentin *et al.*, 1986) : plateau and talus, tree savanna (or woodland), dense and open shrub savannas (with areas of higher erosion on indurated ferrallitic soil), wet woodland, grass savanna and riparian forest. Part of the basin is cultivated (10%) and areas correspond to fallows of different ages.

METHODS AND MATERIAL

Both variables, related to termite mounds and environmental variables have been collected along transects related to the topography (from bottom to the hill top). A total of 5950 m was sampled (width : 10 m), divided into 238 sub-units (250 m²). Each transect is 450-600 m length.

Environmental variables are : soil and vegetation types, gradient, distance from the river. Later on, we shall include soil texture, vegetation biomass and tree cover. Termites related variables are : number of mounds, dead and alive, basal areas and volumes. In first instance, three main ecological categories have been identified (as far as species biology and mound material are concerned) : "*Trinervitermes*", "*Cubitermes*" and "*Macrotermes*" groups.

Data handling, currently in progress, utilize contingency tables, multivariate (principal component and correspondence) analyses.

RESULTS

Preliminary results showed that nearly 50 termite species are encountered on the drainage basin. Among the epigeous nests, several species of different biologies could be found within a few meters : fungus-growing, foraging and humivorous species.

On table 1 are shown the main relationships between mounds and environmental variables, from the chi-square contingency tables. These results give a first insight into termites preferences : the most significant relationships are obtained with the vegetation types. Correspondence analysis evidenced main factors, as slope gradient, tree density, indurated soils.

	Macrotermes				Trinervitermes				Cubitermes			
	area	vol.	live	dead	area	vol.	live	dead	area	vol.	live	dead
Slope	NS	.025	NS	NS	NS	NS	NS	NS	NS	NS	NS	.1
Distance	.005	NS	NS	.025	NS	NS	NS	NS	.1	NS	.025	NS
Soil type	NS	.01	NS	NS	NS	NS	NS	NS	.025	NS	NS	NS
Vegetation	.005	.005	.005	.05	NS	.05	NS	NS	.1	.05	.025	NS
Total.ha ⁻¹	384	77	3	34	5	.7	18	5.7	6.4	1.8	65.7	25.8

Table 1.- Chi-square probabilities values between mounds and environmental variables (NS = non significant at .1 level).

On table 1 are also summarized the overall figures per average ha. Basal areas (dead + alive nests) covered 4% of the drainage basin, and up to 9.7% in grass savanna. Mound volumes amounted to 79.5m³, most of it being Macrotermes mound material. Densities are respectively 24 (Trinervitermes), 29 (Macrotermes) and 100 (Cubitermes). Densities in some vegetation types reached 200 (open shrub savanna).

According to these high figures and to their pattern of distribution, termite nests play certainly a significant role in the water dynamics of the basin. Further studies (rainfall simulations) will be undertaken.

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Fossilized Feeding Pattern of Termites on Wood Remainers of the Tertiary Carbon Formation of Staré Sedlo (CSSR)

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Recently it has been reported (Suess and Rangnow, 1984) on a collection of fossils, brought together by Heinrich Cotta (1763-1844) and deposited since 140 years in the "Museum für Naturkunde" at Berlin. Among various other fossils, this collection contains three heavily damaged pieces of coniferous wood from the tertiary carbon sandstone formation (upper eocene, lower oligocene) of Staré Sedlo (Bohemia, Czechoslovakia). The available evidence points to an attack on this wood by either tree-dwelling ants or termites prior to fossilization. Till now, only one example of a similar petrified nest of termites in coniferous wood has been described in the literature (Abel, 1933). The current study presents first results of our investigations on these fossils.

RESULTS

The fossilized wood stems from an unidentified conifer species. The pieces display characteristic chamber-like excavations, which at first sight appear to be caused by tree-inhabiting ants or by termites. Prehistoric ants were demonstrated as early as 1936 (Brues). The damages caused by ants, however, differ significantly from termite feeding patterns. Wood-dwelling ants characteristically excavate long chambers separated statically and constructionally by latewood lamellae which are left uncut. Termites use similar static principles; they are known, however, to proceed in a different constructional manner. Latewood is known to consist of much shorter lamellae than earlywood, and the chambers of (contemporary) termites usually extend over several annual rings. The essential features, for this reason, are meniscus-like bridges remaining above the chambers. As the cavities in the fossilized wood display exactly this termite-specific construction pattern, they are considered to result from an attack of ancient termites.

An additional proof for a typical termite attack is derived from the characteristic filling of the chambers. It consists of partially preserved meconium-like remainders which are thought to result from an original mixture of excrements, sand and mould particles. Such a mixture is commonly used by soil-dwelling termites for wood-conditioning by a permanent moistening. The filling does not contain any organic skeleton. For this reason, fossilization could not lead to a solid structure, but resulted in a rather loose, easily removable sediment.

Feeding patterns on both recent and fossil wood were compared at 15 times magnification. *Reticulitermes santonensis* DE FEYTEAUD conducts most of its feeding attacks parallel to the annual rings with biting widths of 0,3 mm (Schultze-Dewitz, 1959), leaving some remaining fibre-bundles of primary wood untouched on the latewood. In the fossilized specimens, well conserved earlywood remainders are found on the tangen-

*Dedicated to Prof. em. Dr. H. Weidner on the occasion of his 75th birthday.

tial latewood level, thus displaying a feeding pattern which is essentially the same as the one produced by a recent termite species. A paper with photographs is in press and will be published elsewhere.

DISCUSSION

To classify the termite species involved, microscopical examination and morphological comparison alone will not permit an unequivocal assignment, as the prints of the mouthparts which are left on the wood will not differ significantly within one family. Species or genera which were different from the contemporary ones may have been active during the mesozoic era, and one should not conclude that Reticulitermes was the unique cause of the fossilized feeding traces.

Nevertheless, we assume that soil termites of the genera Reticulitermes or Heterotermes, both belonging to the subfamily Heterotermitinae have produced the fossilized patterns observed. Evidence for this restriction is derived from two features which are considered to be highly characteristic for this sub-family: the overall shape of the chambers in combination with their fillings, which contain organic deposits devoid of structure and are typical for the respective ecosystem.

It has been demonstrated that insects belonging to the order Isoptera have already been existing during the tertiary period. Fully preserved adults or, at least, their wings have been found included into mud or into the resin of trees from the so-called amber forest.

Comparison of fossil termite species with recent ones reveals a surprising degree of species preservation. With the exception of Miotermes, all known fossil genera (Rosen, 1913) are still represented in the recent ones, some of them, however, differing in species designation (Emerson, 1971; Weidner, 1968; 1971). Mastotermes, a very primitive genus, is represented by one recent species only, M. darwiniensis FROGATT, which occurs within the archaic fauna of Australia.

It should be emphasized, that the evidence available within the scope of such a palaeobiological study may not be expected to explain every discrepancy. For the amber forest, e.g., a sub-tropical/tropical climate is assumed. The presence within such an ecosystem of Reticulitermes, of which recent species occur exclusively in moderate climates, seems to be at variance with the apparent absence of the thermophile genus Mastotermes. The remarkable adaptive capabilities of these animals should, however, be kept in mind, as summarized by a statement cited from Weidner (1955): "that termites of the tertiary era had other ecological requirements than the recent ones". The genus Malotermes may be mentioned as an example for such an adaptation to present climatic conditions, since Malotermes flavocollis FABRICIUS is known to occur in southern Europe.

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The Adaptive Significance of Nest Site Selection by Honey Bee Swarms: Calculation of Reproductive Value of Swarms using Colony Life Table Data

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Honey bee swarms are faced with the task of selecting one cavity in which to build a nest, from among the many located by scout bees. But which one? As nest site selection is a collective process among the workers it seems reasonable that they should select a cavity which maximises their own fitness.

Reproductive value (RV) (Fisher 1930), which is the age specific expectation of all future offspring discounted back to the present (Pianka and Parker 1975), can be used to assess the adaptive significance of observed preferences in nest site selection based on the life histories of colonies founded by swarms in cavities of different properties. However, worker bees do not themselves reproduce. The application of RV to nest site selection by swarms, therefore, requires that worker reproduction be quantified indirectly, in terms of the production of kin reproductives by the colony in the chosen nest site. The formula for calculating RV of a normal organism can be simply modified by adding in a relatedness term to give:

$$\int_{t=0}^{\infty} b_t G_{tSR} (l_t/l_0) e^{-rt} dt \quad (1) \text{ limits 0 to infinity as colony is potentially immortal}$$

b_t = reproduction by colony at age t
 G_{tSR} = relatedness between swarm workers and reproductives produced at age t
 e^{-rt} = discount on future reproduction due to population growth
 l_t/l_0 = probability of colony survival to age t

This formula can be simplified by equating e^{-rt} to unity (i.e. number of colonies in local population constant, $r=0$) and by converting it into a sum (i.e. reproduction occurs in a discrete breeding season) to give:

$$\sum_{t=0}^{\infty} b_t G_{tSR} (l_t/l_0) \quad (2)$$

As relatedness between reproductives and the colonising swarm workers declines by half every time the queen is replaced, future reproduction by the colony is rapidly discounted in value to the swarm. By using Formula 2 a life table simulation of swarm RV shows that over half swarm RV comes during the first breeding season, assuming that reproduction is constant each year and that the colony queen is replaced each year. This result focusses attention on cavity properties which enhance survival or reproduction in the first year. Conversely any cavity properties which had long term benefits to the occupying colony would have little importance on nest site selection. In principle, life histories of colonies established in cavities of different properties could be analysed by this method to help test hypotheses concerning the adaptive significance of the preferences shown by swarms for artificial nest cavities of differing volume, entrance area etc. (Seeley and Morse 1978).

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Structure and Origin of Wasp Communities of Warsaw

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In green areas of Warsaw, wasps (Vespidae) consist of only 13 species. They represent merely 50% of the species occurring in homologous natural habitats (Tilio-Carpinetum). Especially the number of species and individuals of solitary wasps is very low in urban habitats. To the contrary, social wasps are expansive in colonizing urban green, especially when subject to low urban pressure. In natural habitats they are represented by 9 species, and in Warsaw 8 species were recorded, including 7 in parks and 2-4 species in other types of green (in housing estates, along streets, in allotment gardens).

The number of individuals of social wasps in the town is almost twice as high as in the homologous natural habitats. Their abundance in urban green is highly differentiated. The most abundant urban community of wasps (in green under low urban pressure) differs from the least abundant urban community (in green under heavy urban pressure) by a factor of 30, whereas in the homologous natural habitats only by a factor of 6.

All the species recorded from Warsaw occur also in Tilio-Carpinetum, but the dominance structure of urban wasp communities markedly differs from that of forest wasp communities. In urban green, two species, *Paravespula vulgaris* and *P. germanica*, reach high densities. In Tilio-Carpinetum, only one species, *P. vulgaris*, is dominant.

Paravespula vulgaris prefers forest habitats, and is abundant in both deciduous and coniferous forests. This species inhabits a much larger spectrum of habitats than *P. germanica*. It is known from all the wasp communities recorded so far in Poland. *Paravespula germanica* prefers open habitats, where it is dominant, and it avoids shaded forest habitats. In Tilio-Carpinetum its proportion is low, if any. Both these species co-occur in wooded but semi-open habitats such as modified suburban woods (on different sites), and some types of urban green (large parks). In such habitats they occur in high numbers and co-dominate.

4.5 Interaction with Other Organisms: Symbiosis, Parasites, Pathogens

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SYMPOSIUM

Symbiosis, Parasites, Pathogens

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A Workerless Inquiline in *Pseudomyrmex* (Hymenoptera: Formicidae: Pseudomyrmecinae)

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Inquilinism in ants is a form of social parasitism where the parasite spends its entire life cycle in the nests of the host species. Inquilines were hitherto known only in three of the 11 formicid sub-families: the Myrmicinae, the Formicinae, and the Myrmeciinae (Wilson, 1971; Buschinger, pers. comm.). In the Pseudomyrmecinae only a temporary social parasite had been reported in the Old World genus *Tetraponera* (Terron, 1969). In addition, there are two records of mixed nests of usually independently living species of *Pseudomyrmex*, indicative of facultative social parasitism: (1) a male, two alate queens, and several workers of *P. pallidus* (F. Smith) associated with several workers of *P. cubaensis* (Forel) in the Bahamas (Wheeler, 1905); (2) a dealate queen of *P. seminole* Ward associated with seven workers of *P. pallidus* in Texas (Ward, 1985). I report here on the discovery of and first biological observations on *P. leptosus* Ward, a workerless inquiline of *P. ejectus* (F. Smith) in Florida.

MATERIALS AND METHODS

I discovered *P. leptosus* queens and brood in nest twigs of *P. ejectus* on 15. and 16. May 1982 at two sites ca. 15 km apart from each other in the vicinity of Gainesville, North-Central Florida: colony #1 in an unidentified twig at the edge of mesic hardwood hammock; colony #2 in several twigs of a *Baccharis* shrub in open seasonal swamp habitat. The colonies were maintained in the laboratory until 14. Dec. 82, with narrow glass tubes serving as artificial nest sites. The ants were fed water, honey solution, and fresh insect meat. The colonies were censused at roughly monthly intervals.

RESULTS AND DISCUSSION

The colonies were originally composed as follows: #1: *P. leptosus*: 2 dealate queens; *P. ejectus*: 43 workers, 60 worker pupae; *unidentified*: ca. 180 prepupae, larvae, and eggs; #2: *P. leptosus*: 2 dealate queens, 3 alate queens, 65 queen pupae; *P. ejectus*: 146 workers, 54 worker pupae; *unidentified*: 8 male pupae, ca. 305 prepupae, larvae, and eggs. When discontinued seven months later, the colonies contained: #1: *P. leptosus*: 2 dealate queens (at least one of them not the original one), 54 alate queens, 6 queen pupae; *P. ejectus*: 21 workers (down from a peak of 76 on 18. July); *unidentified*: 3 male pupae, ca. 135 prepupae, larvae, and eggs; #2: *P. leptosus*: 1 dealate queen, 140 alate queens; *P. ejectus*: 1 worker. There was no brood left in #2, which had essentially disintegrated weeks ago (most queens were clustering in protected places outside the nest tubes), and no worker brood in #1, which nevertheless still appeared strong. During their seven months of laboratory maintenance the colonies had produced at least following numbers of alates (these estimates are conservative, because not all the alates that died or escaped during

intervals between counts are included): #1: 123 P. leptosus queens, 10 males; #2: 310 P. leptosus queens, 42 males (while the queens of P. leptosus can be easily discriminated from those of P. ejectus on the basis of their different color (clear orange as compared to dark brown) this is not the case for the males of the two species. The males sent to P. S. Ward for identification were P. leptosus). Neither of the two P. leptosus / P. ejectus colonies produced any workers.

A third collection of a mixed P. leptosus / P. ejectus nest by B. J. Cole in South Florida on 26. Nov. 84 also contained queens only of P. leptosus and workers only of P. ejectus (Ward, 1985).

P. leptosus is morphologically extremely similar to the sympatric polygynous P. pallidus. P. leptosus queens tend to be smaller, however, have a more broadly rounded head, and differ slightly in the cuticular sculpture of their frons, vertex, postpetiole, and gaster (Ward, 1985).

I observed one important behavioral difference between the queens of the two species: while dealate P. pallidus queens are indifferent to one another and often sit peacefully close together, the dealate P. leptosus queens exhibited agonistic behavior when encountering each other, incl. biting, clinging to and trying to sting their opponent, or fleeing from it. In both colonies, soon only one of the original queens remained. This aggressive behavior, as well as the absence of P. ejectus queens in all mixed P. leptosus / P. ejectus nests collected so far suggests that P. leptosus queens upon invasion kill the host queens that they encounter (for data on the colony structure of unparasitized P. ejectus see Klein, 1986, this volume).

Virgin queens of P. leptosus and P. pallidus exhibited very similar sexual calling behaviors, raising their gasters and, clearly visibly, everting their tergal glands while standing on or near their nest tubes. Like other Pseudomyrmex males, the males of P. leptosus are excellent fliers. Nevertheless, a considerable degree of adelphogamy may occur as indicated by the frequent appearance of new dealate P. leptosus queens in the lab colonies. The highly female biased sex ratio observed in both colonies, too, may indicate adelphogamy and local mate competition.

P. leptosus queens still show some degree of brood care behavior: they groomed and, when disturbed, carried and defended immatures.

It seems beyond doubt, that P. leptosus descended from the independently living polygynous P. pallidus, where joining of established nests by freshly mated queens, i. e. intraspecific parasitization (Buschinger, 1970), seems commonplace. It is easily conceivable, how by joining nests of a different species an adelphogamous P. leptosus ancestor may have become reproductively isolated from the P. pallidus gene pool and developed inquiline characters.

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The Cloning of Selected Fungal Strains; a Factor in the Speciation of Attine Ants

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The taxonomically compact myrmicine tribe Attini comprises approximately 190 species in 11 genera which all cultivate fungi. Several features, including mature ant colony size and the material used as fungal substrate suggest an evolutionary trend (Wilson, 1971). The fungus cultivated by most attines is characterised by gongylidia which constitute the principal supply of larval food (Weber, 1972). Colonies are initiated by claustral foundation, each gyne securing a sample of mycelium from the parent colony (Weber, 1966) and later, using it to establish a new culture. From this, future generations of gynes will in turn derive their own inocula.

The identity of the fungus cultivated by the higher attines remains obscure. Records of taxonomically essential sporophores from mycelia associated with advanced attines are few, and their true origins in doubt. Most recent authors have chosen to use the name Attamyces bromatificus Kreisel, described from the Cuban Atta insularis (Kreisel, 1972). In the absence of sporophore characters this was placed in the Mycelia Sterilia. Examinations of mycelia from attines in 5 genera revealed all to possess typical Basidiomycete septation (Powell and Stradling, 1986). The doubtful occurrence of sporulating stages in the symbionts of higher attines and the ants' vegetative method of propagating their symbionts suggests that these fungi are closely related. They are poor competitors and the germination of contaminant spores is suppressed by secretions from the metathoracic glands (Schildknecht and Koob, 1971).

The present studies were undertaken to investigate the compatibility between and productivity of fungal isolates from different attine species.

MATERIALS AND METHODS

Isolates of attine fungi were obtained from; Trachymyrmex urichi Forel, Acromyrmex octospinosus Reich and Atta cephalotes L. from Trinidad; A. sexdens sexdens L. from Guyana; A. sexdens rubropilosa Forel from both Brazil and Paraguay. All isolates were cultured on PDA plates buffered at pH 5.0 and incubated at 25°C in darkness (Powell and Stradling, 1986). Plates were inoculated with pairs of different isolates representing all 15 possible combinations, each being replicated three times. When the mycelia of both inocula had grown together they were inspected for interactions. A set of 24 replicate plate cultures were established from each isolate alone and incubated for periods of up to six weeks. Total fungal fresh weight, and gongylidia biomass were determined for four plates from each isolate at weekly intervals. Estimated worker populations for mature colonies of each species were obtained from literature.

RESULTS

No interactions were observed in any of the paired tests. Increases in fresh weight shown by the separate cultures indicated differences in productivity between all six isolates by the sixth week of incubation. Total fungal production was reflected in the "crop" yield of gongylidia. Comparison of gongylidia production after six weeks incubation with estimates of mature colony worker populations for the respective ant species reveal a strong correlation, $r = 0.99$ ($p < 0.001$) described by the linear regression; population = $-225874 + 70157 \times$ gongylidia biomass.

DISCUSSION.

The suggestion that higher attines cultivate the same species of basidiomycete fungus is supported by the absence of obvious interactions in paired cultures. Not only is sporulation by these fungi doubtful but ant secretions militate against propagation by spores. We therefore propose that not only do different species of attine ants cultivate the same species of fungus but that through their vegetative method of propagation these cultures constitute fungal clones which may be shared by more than one ant species.

There are however, significant differences in their productivities which correlate strongly with the maximum colony populations of the ant species.

Food production (gongylidia) might be expected to have significant importance to reproductive success. Larger worker populations would allow an increased foraging range and thus facilitate a expansion of the trophophoric field. Thus the acquisition of a more productive food crop would confer a selective advantage in permitting the exploitation of richer and more abundant substrate resources and would therefore constitute an important factor in attine niche separation. The only source of more productive fungal strains would be through somatic mutation. We suggest that ancestral selection of high yielding somatic mutants from their cloned fungal symbionts was a major factor in the speciation of the higher attines allowing niche separation through selection for increased queen fecundity and colony population.

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Biological Control Agents of Fire Ants in Brazil

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In February 1984, the U.S. Department of Agriculture and the Empresa Brasileira de Pesquisa Agropecuária jointly established a laboratory at the EMPA-MT Agricultural Research Station in Cáceres, Mato Grosso (MT), Brasil, for the purpose of surveying for and evaluating the natural biological control agents of fire ants, primarily *S. invicta* Buren. Standard size samples of 2 1/2 liters of tumulus excavated from fire ant mounds are placed in buckets. The ants and myrmecophiles are separated from the soil by floatation with water (Jouvenaz et al., 1977). Individual ants and aqueous mass extracts of ants are examined microscopically for pathogens (Jouvenaz et al., 1977). Myrmecophiles are identified and preserved.

Since April 1986, 1000 fire ant colonies have been examined for natural enemies. All of the colonies were collected within 200 km of Cáceres, except for about 100 colonies that were collected near Campo Grande, Mato Grosso do Sul. Single colonies may commonly contain several species of pathogens and/or myrmecophiles. No correlation has been found between the occurrence of any of the pathogens and/or myrmecophiles.

The total numbers and other parameters for each pathogen and myrmecophile collected during the 21-month survey (July 1984 through March 1986) are given in Table 1. Of the 1000 colonies examined, 757 contained at least one species of pathogen and/or myrmecophile. The known biology of these pathogens and myrmecophiles is discussed, respectively, by Jouvenaz (1986) and Wojcik (1986). The microsporidians, *Thelohania solenopsae* and *Vairimorpha* sp., are known only from South America and infect brood and adult ants. The neogregarine, *Mattesia geminata*, has been found in *Solenopsis* spp. nests in North and South America and infects only immatures. The undescribed nematode (Mermithoidea: Tetradonematidae) was found for the first time in February 1985; it infects brood and adults. Several other pathogens, including virus-like particles, an undescribed neogregarine, a fungus, and possibly a bacterium, have been reported from fire ants in Brasil but have not been collected during this survey.

The scarab beetles (genus *Martinezia* and the unidentified *Thysanura* are predacious on fire ant brood. Nothing is known about the biology of the unidentified histler and chrysomelid (case-bearing larvae) beetles or the lygaeid bugs. The ectoparasitic larvae of the wasps of the genus *Orasema* (Eucharitidae) cause malformation and death of fire ant pupae. The adult wasps lay their eggs in plant tissue causing cosmetic damage. The workerless obligate parasitic ant, *Labachena* sp., has been collected once during this survey, and our attempt to colonize this species in the United States was unsuccessful. One phorid fly puparium was dissected from an alate female fire ant. The unidentified millipedes are thought to be scavengers. Other myrmecophiles associated with fire ants in South America (Wojcik, 1986) have not been collected during this survey.

In the coming year, we will continue the pathogen and myrmecophile survey. We will emphasize the collection and colonization of the nematode and the *Labauchena* sp. in order to evaluate these organisms for possible introduction into the United States. The ultimate goal of our project is to establish, in the United States, a complex of specific natural enemies as a biological control component of an integrated pest management program for fire ants.

Table 1. Summary of collection records for pathogens and myrmecophiles found in 1000 fire ant colonies in Brasil.

Organism	Number of colonies with organism	No. of specimens	Avg. no. per nest	Range	Median	Mode
Pathogen						
Thelohania	22					
Vairimorpha	46					
Mattesia	64					
Nematode	7					
Myrmecophile						
Scarabaeidae	213	637	3.0	1-31	2	1
Histeridae	189	392	2.1	1-12	1	1
Chrysomelidae	2	3	1.5	1-2	1.5	-
Eucharitidae	413	7225	17.5	1-598	4	1
Formicidae	1	101	-	-	-	-
Lygaeidae	16	28	1.8	1-5	1	1
Thysanura	17	30	1.8	1-12	1	1
Diplopoda	111	554	5.0	1-48	2	1
TOTALS	757	8970				

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Microorganisms in the Gut of Ants of the Tribe *Cephalotini*: Location and Relationship with Intestinal Structures

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The gut of ants is very uniform in its morphological and histological aspects and its organization is much the same that of the Hymenoptera group (Caetano, 1981 and 1984). A few differences have been detected by Eisner (1957), Caetano (1981 and 1984), and Caetano and Lage Filho (1982) in the proventriculus bulb, the oesophagus and the hind-gut. The objective of this paper is to clarify the nature of a fibrous material present in a dilated and "dark portion" of the ileum in the tribe *Cephalotini*.

MATERIALS AND METHODS

The ants used in the present study were *Paracryptocerus pusillus* and *Cephalotes atratus* (Myrmicinae). Portions of the gut were fixed in 2,5% glutaraldehyde in sodium cacodylate buffer, pH 7.2, with 4% sucrose during 2 hours, washed twice in fresh buffer (15 minutes each), post-fixed in 1% osmic acid in the same buffer; washed twice again with fresh buffer and dehydrated in a series of increasing ethanol concentration, and embedded in Epon (1:1 Luft) via propylene oxide. Sections were made with glass knives in a Porter Blum MT2 U1 Ultramicrotome and contrasted with uranyl-acetate and lead citrate. The material present in the hind-gut was dissociated in 1% phosphotungstic acid and collected on copper grids, dried up and observed in the electron microscope.

RESULTS

The morphological and histological features of *C. atratus* and *Z. clypeatus* were obtained from Caetano (1984). The Dark portion of the hind gut of *Cephalotini* ants is filled by fibrillar material and the circular sheath of muscle is considerably developed in this portion. Under the EM, however, this musculature is similar to the one of other regions of the hind-gut. Its greater volume is due only to the increased number of muscular fibers on the sheath. The fibrillar material appears to be a mass of microorganisms. The negative contrastation showed that this microorganisms are fungi and bacteria. The fungi are non-septed, exhibiting considerably long hyphae with countless vacuoles. The bacteria are of various types, with bacillus predominating. Microorganisms were also observed in the ventriculus, but in different densities from the proximal to the distal portion. The proximal portion has no microorganisms while in the distal portion large amounts were detected in the lumen and among the microvilli of the digestive cells.

DISCUSSION AND CONCLUSIONS

The presence of an elaborated proventriculus bulb is an evidence for a passive mechanism to retain the food in the crop for a long period (Eisner, 1957). *Cephalotini* ants have a proventriculus bulb finely

cracked with hawthorn cuticle and weak muscle. This suggests low motility and no passage of solid particles, so the food stuff used by these ants is liquid. The presence of microorganisms in the gut of insects is well known. But in most insects, the microorganisms are present in the hind-gut, where they are free from the action of the digestive enzymes. In Coxotermes and Heimitermes, bacteria are observed in the posterior portion of the ventriculus (Grasse & Noirot, 1959). The presence of microorganisms in this region suggests two hypotheses: 1º in most insects the distal portion of the ventriculus is the region where digestion actually occurs and the microorganisms are there to aid the process: 2º microorganisms may be frequent in this region, but after digestion in the pouch existing in the hind-gut, the contents of the pouch may be sent back to the ventriculus for absorption. The permanence of the microorganisms in this region would be limited, since they would soon be degraded by enzymes. However, no sign of microorganisms degradation was observed in the ventriculus. The microorganisms are found in the hind-gut of termites, cockroaches and some coleopterans. Catopidae coleopterans have a pouch (Strambi and Zylberberg, 1966 and 1972) similar to that observed by us in the hind-gut of the ants. Like in the ants, this region of the gut has a considerably reduced epithelium and a strong circular musculature, the lumen being completely occupied by filamentous bacteria. In termites, however, the circular musculature found in the microorganism pouch is little developed. We believe that this strong circular musculature found in ants may have the function of better promoting the mixing of food with microorganisms and/or to promote their later reflux towards the ventriculus. In conclusion, we suggest that these microorganisms have no auxiliary function in digestion, but they use the food residues, left behind by the ants, supplying in turn alimentary complementation for their hosts through the products which they eliminate into the gut. Food residues and microorganisms then reflux towards the ventriculus for absorption.

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Intestinal Symbionts, Microorganisms, and Abdominal Trophallaxis in the Neotropical Myrmicine Tribe *Cephalotini* (Hymenoptera: Formicidae)

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INTRODUCTION

Cephalotini is a well defined tribe of arboreal myrmicine ants restricted to the Neotropics, that includes 4 genera. Wheeler (1984) reported abdominal trophallaxis in *Procryptocerus scabriusculus*, primarily between just emerged callows and older workers, in contacts that lasted as long as 30 min., suggesting that the tribe may have undiscovered dietary peculiarities. Caetano & Cruz-Landim (in press) showed that the posterior end of the ventriculus and specially a hind gut dilatation are filled with a bulky mass of microorganisms, including various kinds of bacteria and less abundant fungi, but no protozoaries.

In this study we report observations on the food preferences of *Zacryptocerus pusillus*, along with anatomical figures of this species, *Cephalotes atratus* and *Eucryptocerus* sp. and histological and ultrastructural figures of *C. atratus*.

MATERIAL AND METHODS

Five colonies of *Z. pusillus* were collected in Corumbatai, SP, Brasil, transported to argyl or Gypsum nests and submitted to food deprivation for 5-8 days before being allowed to collected among 21 different food items available in separate arenas. Workers of *C. atratus*, collected at Rio Claro, SP and that of *Eucryptocerus* sp., collected at Serra dos Carajás, PA, Brasil were transported frozen to the lab, dissected and their alimentary canals immersed in physiological solution. Portions of *C. atratus* were: 1. prepared for examination under E.M. following standard procedures or 2. dissociated in 1% phosphotungstic acid, and the dispersed material collected in copper grids, dried up and observed.

RESULTS AND DISCUSSION

Our data on food preferences of *Z. pusillus* indicate that these ants are omnivorous, with slight preference for sugar-rich items, though attracted to several kinds of food items.

We observed in callow workers the hind gut dilatation already formed, though completely free of microorganisms and light-colored. The circular muscular sheets around this portion are also stronger than in other regions of the hind gut, suggesting differentiation at an early embriological stage.

The extreme specialization of cephalotine digestive tube may be related to the diversification in their food habits and possibly related to the diversification of intestinal microorganisms symbionts.

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A Review of the Termitophilous *Coleoptera* Associated with *Hodotermitidae* (Isoptera)

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The last review of the Arthropods found with *Hodotermitidae* was written in 1970 (Kistner). In these intervening years many collections were made in South Africa by both Kistner and by the late W.G.H. Coaton and J.L. Sheasby of the Plant Protection Research Institute in Pretoria. These collections have yielded the first termitophiles from a nest of *Hodotermites mossambicus* (Hagen) as well as a far larger collection from the nests of *Microhodotermites viator* (Latreille) than was studied during the preparation of the 1970 paper.

The collection of the termitophiles is effected by digging up the nests, scooping out the softer interior and then sorting this material over plastic dishes in the field. Most termite activity is in the soft material made of soil and carton in the top part of the nest where the queen is located. The queen is capable of moving about in the upper nest and is not confined to a royal cell. For further descriptions of the nests and their distribution in South Africa and Namibia, see Coaton and Sheasby's (1974) paper. The termitophiles are readily apparent in the plastic dishes and they were then transferred into Petri dishes in which some moist toweling was placed together with some termites and nest materials from the nest. This is easier done with *M. viator* than with *H. mossambicus* because *M. viator* makes mounds over their nests in certain areas of South Africa which makes them readily apparent. Digging in hard dirt is better motivated when one knows that the nest will indeed be found. The nests of *H. mossambicus*, on the other hand, are much deeper in the soil and the only indications on the surface are frequent foraging holes which may be meters away from the central nest. The single sample was secured from a nest after it was dug into by an aardvark. After studying the interactions of the termitophiles with the termites, specimens were preserved in Isopropanolic FAA and then transferred to 80% ethanol. Such specimens were then sectioned later in the laboratory to see what if any unique glands are present in the specimens.

The collections yielded new records of *Termitotelus neoschultzei*, *T. niger*, *T. sheasbyi* and *Hodoxenus sheasbyi* as well as a new species, *T. maculosus* from nests of *Microhodotermites viator*. A new genus and species, *Neotermitotelus hartwigi* was found in the nest of *Hodotermites mossambicus* the first termitophilous staphylinid recorded from these nests. Also collected from nests of *M. viator* was a new species of Throscidae, *Neocrowsonia viatoricus*, a highly derived beetle and the first termitophilous throscid recorded.

RELATIONSHIPS

The relationships of the species of *Termitotelus* to the new genus and to *Atheta* were studied cladistically using the Wagner algorithm present in the PHYSYS program written by James S. Farris and adapted for the California State University central Cyber by Farris, Richard A. Pimentel, and James D. Smith.

DISCUSSION OF THE RELATIONSHIPS

The new genus and species, *Neotermitotelus hartwigi* clusters out as closely related to the *Termitotelus coatoni* - *neoschultzei* - *schultzei* complex. However, we consider the uniquely shaped mandibles adequate justification to separate the species into its own genus. The cladistics indicate that *Neotermitotelus* has been derived from *Termitotelus* stock. *Termitotelus sheasbyi* also clusters out as ancestral to the rest of *Termitotelus* and *Neotermitotelus* and there might be reasons found in the future to assign it to its own genus. Because of the distance from *Atheta* implied by the lack of shared derived characters and the absence of such typical "Athetoid" characters as a bifid glossa, we may wish to rethink the relationships when the Aleocharinae are better known.

If the cladistic relationships derived here represent the phylogeny of this small termitophilous group, then the most primitive species of the Termitotelina are found with the more derived termites as *Microhodotermites* is derived from *Hodotermites* according to Ahmad (1950) and Krishna (1970). This could simply mean that the termitophiles evolved slower with *Microhodotermites* than with *Hodotermites*.

BEHAVIOR AND GLANDS

The behavior of 2 species was observed by Kistner and Clover in the field, *Termitotelus maculosus* and *T. neoschultzei*. Neither species lasted long in close proximity to the termites. The above 2 species did not differ in their behavior. When in contact with the termites, they were frequently seen to jump 2-3 inches from 1 spot in the Petri dish to another. Ordinarily, the beetles avoided contact with the termites by running away or by jumping away. We observed the termites coming in contact with the beetles 27 times. What happened on 3 occasions, was that the beetles raised their abdomens and the termites were seen to draw back. This allowed the beetles another chance to get away. The interpretation of this is that these termitophiles avoid the termites and they can do so as long as they can get free and move away. They can use their defense gland and/or their stiff bristles to cause the termites to draw away. Once these options are no longer functional, the beetles are killed by the termites. Histological sections of the beetles were investigated which revealed a large defense gland of the type typical of *Drusilla canaliculata* as described by Pasteels (1968) and Araujo (1978). Sections of *Termitotelus niger* revealed numerous single celled gland cells in the epidermis. However these are particularly concentrated beneath the anterior border of sternite VII in this species. No other species of *Termitotelus* shows the concentration of pores or cells in this particular area.

The behavior of *Hodoxenus sheasbyi* was also investigated in the field using the same techniques. When isolated with termites, this species seeks out the termites, antennates them, and offers the tip of its abdomen to them. Workers were seen to run their mandibles down the abdomen, ending and lingering near the tip. In most instances, the worker then quits and the *Hodoxenus* walks away. However, on numerous occasions, the worker actually followed the termitophile, hanging onto the tip of the abdomen as though drinking something from it (65 observations). We observed *Hodoxenus* drinking proctodeal fluid at least 7 times and managed to photograph this once. The rogatory behavior is a sequence as follows: The beetle runs along side of the termite worker near and even through their legs. At some point the termite stops, the termitophile then antennates the worker and moves its abdomen toward the worker at the same time. The worker may or may not move its mandibles over the abdomen of the beetle, but whether it does or not, it will extrude a clear liquid from the proctodeum (proctodeal food) which the beetle will then imbibe. In doing many of these studies, we would isolate each specimen in a small vial with some termites. After bring specimens back to our hotel, a small larva was noted in the vial with a female *Hodoxenus*. This small larva is a aleocharine larva of an early instar, which may indicate that the beetles are either larviparous or that the eggs hatch very rapidly after being laid. No chorion was found. The larva awaits description after more specimens are secured.

This species is far more integrated into the termite society than any *Termitotelus* species studied and survived long contact with the termites during our hours of observation. There are many pores and many single celled glands associated with abdominal segment IX as well as flattened, modified setae. The function of the branched setae is unknown.

For further details of this study, see Kistner & Abdel-Galil's (1986) paper.

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***Termitomyces albuminosus* and its Relation to *Xylaria nigripes* Associated with *Odontotermes obesus* (Termitidae; Macrotermitinae)**

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As reported for *O. microdentatus* 3 destroyed mounds of *O.O.* produced X.N. and cultures from about 900 spheres of *Aegerita duthiei*, about 650 combs in bell-jars from 165 mounds, about 500 rain-subjected combs from 56 mounds, and search for spores of X.N. here yielded the same results. Additionally, in 58 out of 132 mounds columnar primordia of T.A. grew. In polythene bags they quickly expanded their caps while cross-sections also revealed perithecial walls. Cut primordia, when exposed to the rain grew out into rose-coloured ascocarps with spores of X.N., indicating that absence of the spherocysts containing universal veil allowed upright growth of bell-jar induced stromata and homology of conidial state of X.N. with hymenium of T.A. Some bell-jar induced conidial stromata showed a further modification of the already 4- or 2-sterigmate vesicles to such with mostly 4 curved and spore producing ones. Simulating u.v. applied to upright growing stromata of fast-growing strains of X.N. led to forms suggesting sterile T.A. This agaric we now regard as an excessively developed conidial state of "X.N.", modified to this habitus by the u.v. of A.E.D. and capable of taking over meiosis in a defined type of its otherwise asexual form. So previous failure of growing T.A. and contradictory taxonomical results due to an ascus-basidium based system are explainable. Looking at nest-independent taxa of traditional Higher Fungi as possibly derived from A.E.D. appears to be an attractive working hypothesis.

Nitrogen Fixation by *Nasutitermes* and *Velocitermes* in Venezuela

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Previous studies on *Nasutitermes* species in Costa Rica rain forest provided unequivocal evidence for (1) an inverse correlation of food nitrogen with fixation rates, (2) uptake of worker-fixed ^{15}N by soldiers, (3) importance of natural faunation for maximal fixation rates during nitrogenase analyses, and (4) unexpectedly high fixation rates in soldiers which can account for nearly 25% of the colony's annual nitrogen budget. The present data examine the differences in nitrogenase activities in sympatric *Nasutitermes* sp. and *Velocitermes* sp. from savannas of the Venezuelan llanos. Although both grass-collecting, mound building species co-occur in the same field site, *Velocitermes* nitrogenase rates are less than 5% of those of *Nasutitermes* for both soldiers and workers.

On Some Taxonomically Puzzling Phenomena Observed in the Fungus Associated with *Odontotermes microdentatus* (Macrotermitinae)

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Combs from 1 of 2 destroyed mounds of O.M. R. et S.-S. had produced 62 ascocarps typical of *XYLARIA NIGRIPES* Kl. Microscopically, each of some 450 combs from 81 healthy nests only offered the same type of mycelium forming its spherical bodies (= *AEGERITA DUTHIEI* Berk., Moniliales), generally accepted as imperfect form of the agaric *TERMITOMYCES* H. About 700 transfers of single spheres or few hyphae to malt extract agar, immediately after comb extractions, nearly always yielded very slowly growing, yeast-like, identical and pure cultures from the very start, reproducing shape and structure of the inoculated spheres, usually dying with desiccation of the agar, but otherwise, from 4 to 6 months after inoculation they resulted in fast growing but undiagnostic mycelial cultures blackening the agar. They appeared identical with cultures obtained from hymenial tissues and basidiospores from *T. MICROCARPUS* H. via the same yeast-like phase or from hyphae taken from sterile or only conidia bearing stromata of X.N. induced from combs in a laboratory. Co-inoculated ordinary moulds used to overrun AE. D. within a few days as did fast growing strains of X.N. In the middle of the rainy season the spheres on combs close to the surface of 3 mounds rapidly multiplied, causing workers to detach all of them, and to at first deposit them on the walls of comb chambers, and then on the outer surface of the mounds. Within 24 hs layers of innumerable spheres gave rise to carpets of T.M. About 150 combs from 33 mounds, either dotted with spheres or already entirely deprived of them but still covered with the usual mycelium, when placed in a sun-protected site to get them soaked from daily rains, very fast and regularly developed an abundance of upright, simple or branched ascigerous stromata wholly or partially lacking a black rind but with ascospores like those of X.N. and with spherocysts of AE.D. in their cortices. Attempts made to trap ascospores of X.N. or to locate them in the combs completely failed. Laboratory treated combs from 81 mounds also invariably and quickly produced an abundance of the well-known upright stromata with black rinds and white apices, sterile or only conidia bearing. Ascospores and tissue hyphae from typical X.N. gave very fast growing mycelia on sterile oats, leading to the sight of laboratory induced stromata or, when such a sterile culture was buried in soil, produced the small type of stromata with conidia only. Cap-like formations on cartilaginous stipes sometimes obtained we now interpret as sterile and imperfectly developed forms of the agaric. Since X.N. is the ascigerous form of the fungus of O.M. the development of T.M. from the spheres is here concluded as the expression of the very variable conidial state of X.N. in that case when there is no meiosis in asci.

Methane and Other Metabolic Gases Produced by Termites

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The concentration of methane in the earth's atmosphere is increasing by 1 to 2 percent each year. Since this gas absorbs radiation in the infrared spectrum it is an important greenhouse gas. Most atmospheric methane is biogenic, produced during the anaerobic decomposition of organic matter. Sources include aquatic benthic sediments (including rice paddies), ruminant mammals, and termites. In the ruminants and termites the methane is produced by anaerobic fermentation in the gut, carried out by a specialised symbiotic flora and fauna.

Aerobic metabolism produces carbon dioxide as its main waste product. Anaerobic metabolism produces methane mixed with a small amount of carbon dioxide. Thus the ratio of methane-to-carbon dioxide in expired gases is an indicator of what proportion of the total metabolism is carried out aerobically by the termites themselves and what proportion depends on the gut symbionts. All termites eat food of vegetable origin, but they utilise a wide variety of food sources, and this may influence their metabolic regimes. The ratios of methane-to-carbon dioxide respired by very different trophic groups of termites were studied.

Fungus-growing termites dominate the termite fauna in large parts of the Old World. As well as intestinal symbionts, they depend on external symbionts in the form of fungi which are cultivated on fungus combs inside the nest. One species, *Macrotermes subhyalinus* (jeanneli?) is particularly suitable for study because the whole output of metabolic gases is vented through a single tall chimney. Air flows out continuously at a rate of about 150 litres per minute from a mature nest. The temperature of the outflow is constant at 29°C, the humidity is super-saturated, the carbon dioxide concentration is about 8000 ppm and the methane 12 ppm. Thus in one day a nest emits approx. 200,000 litres of air containing 1700 litres of carbon dioxide and 2.5 litres of methane. It also loses 6 kg of water, surprising in a species that lives in arid lands. The methane-to-carbon dioxide ratio in the outflow is about 0.15%, while gases respired by isolated termites had 0.5%. This implies that 1/3 of the total metabolism is carried out by the termites themselves and 2/3 by the fungus combs.

A soil-feeding termite, *Cubitermes ugandensis*, was studied for comparison. The nest has no ventilation system, so samples were collected in enclosures sealed onto the mound surface. The methane-to-carbon dioxide ratio is about 1%. *Cubitermes* has an extensive gut flora but does not cultivate fungus combs, so a larger proportion of the overall nest metabolism is anaerobic in this case. The weight-specific production rate for methane is extremely high when allowance is made for the soil in the gut, which comprises 65% of the dry weight of the worker termites.

The Pathogenicity of the Entomogenous Fungus *Metarhizium anisopliae* to *Cryptotermes brevis* and its Intestinal Protozoa

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The pathogenicity of one strain of *Metarhizium anisopliae* to workers of *Cryptotermes brevis* and its intestinal protozoa have been studied. Two experiments have been involved. In the first, the survival of the termite individuals was visually observed and recorded daily. In the second experiment, three individuals were dissected every two days to count the number of their intestinal protozoa. The two experiments were replicated six times. In addition, starvation control with two replicates was also carried out.

The results indicated that the entomogenous fungus *Metarhizium anisopliae* is highly toxic to *Cryptotermes brevis* and the time (in days) taken to reach more than 50% mortality was five days. The results obtained showed also that starvation is more effective on the protozoa population than the pathogenic fungus.

Tool Use by a Termite Predator

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In the Neotropical Region lives an assassin bug, Salyavata variegata Amyot and Serville (Reduviidae), that preys on termites of the Genus Nasutitermes. It has evolved a baiting type of predation (involving tool use) that takes advantage of three Nasutitermes characteristics: 1) the concentration of large populations within carton nests, 2) the periodic and spontaneous opening of the nest surface for nest expansion, and 3) the consumption of their own dead and injured. A juvenile bug, camouflaged with carton crumbs scraped from the nest, captures a termite at a nest opening. With its beak it injects its prey with toxins and histolytic enzymes and sucks out its body contents. The bug then dangles the spent carcass over the hole, among the repairing worker termites, until one of them grasps it and tries to pull it into the nest. The bug backs slowly away, drawing the tenacious worker from the nest's protection. It then releases the carcass bait, seizes the new victim, injects it with poisons, and extracts its body fluids. Each victim serves as bait for the next until the bug is satiated. Sometimes the carcass is used mainly as a shield to enable the bug to lean into a nest opening, undetected and unscathed, and to hook out a fresh victim. Only third, fourth, and fifth instar bugs have been observed to bait, but all stages, including the adult, capture and eat termites. When caged with various termite castes and stages, all five juvenile instars, as well as the adult, preferred large workers over small, and small workers over soldiers.

Sacbrood Virus Disease in *Apis cerana indica* F. in South-East Asia

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Sacbrood virus disease of the European honeybee *Apis mellifera* L. has now been reported in Indian hive bee *Apis cerana indica* F. Since 1976, it has spread from Thailand to Burma, Nepal, Pakistan, north-east/west India. Incidence and severity of the disease have been increasing at an alarming rate, killing more than 95 per cent of the colonies, rendering millions of dollars of equipment useless and thousands of beekeepers jobless. The disease has also adversely affected pollination services by native honeybees. Keeping in view the economic loss to the apicultural industry in India and neighbouring countries, surveys for disease incidence, virus identification and screening for resistance in *A. cerana* colonies were carried out.

MATERIALS AND METHODS

Diseased larvae, pre-pupae and pupae of *A. cerana* affected with sacbrood virus were collected from different apiaries of northern India and deep frozen (-25°C). Extraction and purification of the virus was done by slightly modifying the procedure of Bailey et al. (1982). Ten diseased larvae/pupae (ca. 2 gm) were ground in 20 ml of 0.5 M potassium phosphate buffer (PB) pH 8.0, containing 0.02 per cent diethyldithiocarbamate and 0.02 M ethylenediamine-tetra-acetate plus 2 ml of CCl_4 . The slurry was filtered through a piece of cotton and the filtrate cleared by centrifugation (500 g, 10 min at 5°C). The virus in the supernatant was further clarified (8000 g, 10 min, 5°C) and finally sedimented by ultracentrifugation (100,000 g, 2 hrs. at 5°C). A cleaner preparation was obtained by resuspending the pellets in PB and repeated differential centrifugation.

Three antisera were obtained from Rothamsted Experimental Station, England. For immunodiffusion tests, a gel consisting of 0.75 per cent purified (Difco-Bacto) agar, 0.05 M EDTA, 2 per cent NaCl and 0.02 per cent NaN_3 in PB (0.05 M, pH 8.0) was prepared. The purified virus (ca. 1 $\mu\text{g}/\text{ml}$) was tested against specific antisera to sacbrood, Kashmir and chronic bee paralysis viruses. For electron microscopy, collodion coated copper grids were floated on a drop of purified virus, washed in distilled water several times, sucked with filter paper, stained immediately by floating on freshly prepared 2 per cent aqueous uranyl acetate for 1-2 min and air dried. The grids were screened under a JEOL 100S TEM at 100 KV.

For screening of resistance, different colonies of *A. cerana*, healthy/without any symptoms, collected from the disease affected apiaries in different parts of northern India, were fed/sprayed with the virus suspension (1:300 v/v) in 50 per cent sugar syrup.

RESULTS

Symptoms: Symptoms of the present sacbrood disease were similar to

those reported for *A. mellifera* from Europe and North America (Goch-nauer, 1978), including mottled appearance of combs with sunken and perforated brood cappings (Fig. 1). Larval colour changed from white to yellow or greyish and finally to black. Diseased larvae were generally odourless, without ropiness, lying straight in the brood cell; dead larvae formed greyish black scales. Key symptom of this disease was the 'saclike' appearance of pre-pupae. Diseased colonies showed abnormal behaviour such as no coverage of diseased brood by nurse bees, absconding, reduced prolificness and brood, increased aggressiveness and reduced efficiency to throw out the dead brood.

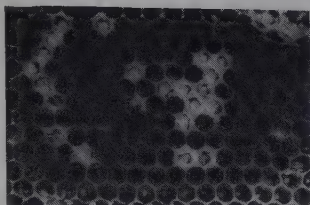


Fig.1. Sacbrood virus diseased comb

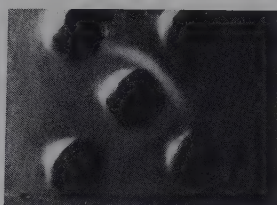


Fig.2. Positive sacbrood immunodiffusion test

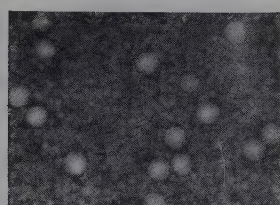


Fig.3. Sacbrood virus particles; X1,17,500

Virus Identification: The virus isolate under study developed a single sharp, positive precipitin band in gels only with a specific antiserum against sacbrood virus (Fig. 2), but not with antisera against Kashmir bee and chronic bee paralysis viruses. The reactions were greatly influenced by temperature. For example, a precipitin band was observed within 4-5 days at 10-15°C, it required, however, only 24 to 36 hrs at 24-25°C. Electron microscopy of both crude and partially purified preparations revealed isometric virus particles of 30 nm diameter (Fig. 3). Bailey et al. (1982) observed similar virus particles in diseased larvae/pupae of *A. cerana* in Thailand.

Tests for Resistance in *A. cerana* and *A. mellifera* colonies: Different preventive or control measures (requeening of the colonies, antibiotic treatment, destruction of infected colonies, migration of colonies to disease-free areas), which were recommended to check the spread of this disease in *A. mellifera* colonies did not help in controlling this disease in *A. cerana*.

At this stage, the only effective measure appeared to be the development of resistant *A. cerana* strains by selection/breeding.

In each affected apiary, about 2-5 per cent of the colonies were found resistant. These colonies were collected at one apiary and were tested by feeding the virus in 50 per cent sugar syrup. All colonies developed symptoms within 4 days of virus feeding and the extent of the infection was more pronounced in the mother queen colonies than in the daughter queen colonies. Such colonies recovered from an infection within 30 days, depending upon the severity of the infection, and the extent of infection, and recovery from the disease increased with the passage of time. In north India, the European honeybee, *A. mellifera*, was introduced for greater honey production. Both *A. cerana* and *A. mellifera* are now kept in the same area. So far, all *A. mellifera* colonies have escaped natural infection by this virus disease and also upon feeding them with the virus suspension.

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The Genus *Volucella* (Diptera: Syrphidae) as Commensals and Parasites in Bumblebee and Wasp Nests

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Most European species of the Genus *Volucella* (Syrphidae) deposit their eggs into the nests of bumblebees or social wasps. The larvae of *V. zonaria*, *V. pellucens* and *V. inanis* live mainly in the nests of *Paravespula vulgaris* and *P. germanica*, while *V. bombylans* is found in the nests of different species of *Bombus*. The adult flies feed on pollen and nectar, whereas the larvae are believed to be scavengers and parasites of their host's brood (Kistner, 1982).

The larval development of *V. inanis* and trophic relations with its host are described. The larval feeding behavior and location within the host nest, as well as morphological features of mouthparts and digestive tract of the different *Volucella* species are compared.

Materials

Adult flies from the field were olfactorily stimulated to egg-laying in the laboratory. Larvae of the different *Volucella* species were reared in nests of their specific hosts, as well as in the laboratory.

Results

Immature *Volucella* are typical maggots of the Cyclorrhapha as first described by Künkel d'Herculais (1875). All three larval stages look quite similar in *V. bombylans* and *V. pellucens*, whereas in *V. inanis* they are strikingly different from each other in morphology and behavior.

Larval instars of *V. inanis*: L1: length (after hatch) 1mm; oval; prolegs armed with a single row of three large claws. L2: max. 7mm; dorsoventrally flattened and protruding lateral margin, dorsal cuticula smooth; prolegs with two rows of claws (8, 10); (Fig. 1). L3: max. 18mm; oval; prolegs with a single row of 10 claws; long posterior respiratory process terminal.

The eggs of *V. inanis* are laid on the external envelope of the wasp nest (ovary contains about 600 eggs). After 5 days (24°C) the larvae hatch, they are quite mobile and migrate into cells containing living wasp larvae. The 2nd -flattened- instar larvae are likewise in cells with 4th and 5th instar larvae of the wasps (Fig. 1); if combs are exposed to light the larvae emerge, leave their cells and quickly look for another cell. Seated in the cell the larva's prolegs always are in contact with the cell wall. Preliminary tests with radioactive tracers indicate that the 2nd instar larva probably feeds either on the saliva or the haemolymph of the wasp larva. Damaged wasp larvae were never observed. The 2nd instar larva remains inside the cell while the mature wasp larva spins its cocoon. Afterwards the *V. inanis* larva moults into the 3rd instar, which completely sucks out the wasp pre-pupa (Fig. 1). Three days after capping, the larva may be fully-grown. The mature larva leaves the cell and burrows into the ground beneath the nest.

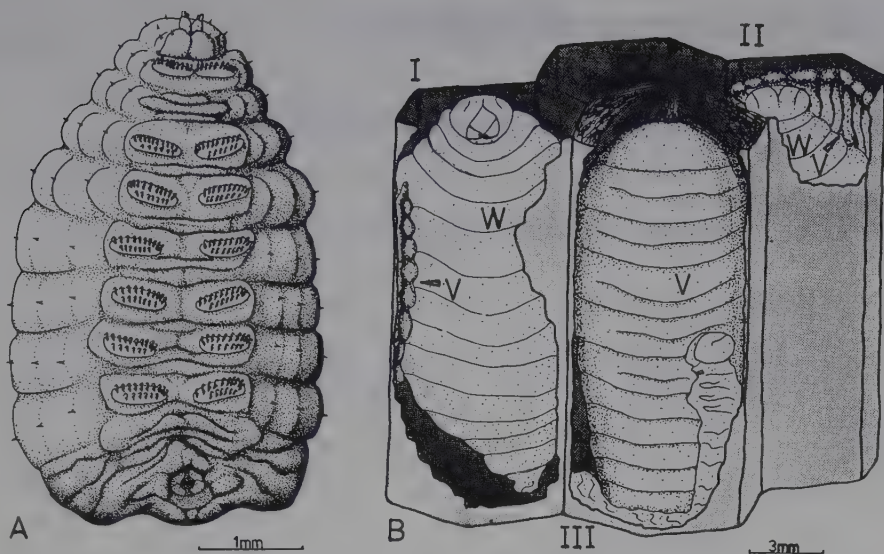


Fig. 1. -- A: *V. inanis* 2nd instar larva, ventral (schematic); B: cells with wasp brood and *V. inanis* larvae. I+II: 2nd instar *V. i.* larvae (V) in different positions with wasp larvae (W). III: 3rd instar *V. i.* larva (V) and the integument of a sucked out wasp pre-pupa.

The ovary of *V. bombylans* and *V. pellucens* contains about 100 and 70 eggs, respectively. The larvae are found beneath the host nests. According to some observations, the larvae attack their host brood, but usually this occurs only in the final stages of nest development. It is possible to rear the larvae of *V. bombylans* and *V. pellucens* outside a host nest on pollen or dead brood, respectively, whereas *V. inanis* larvae die within a few hours outside the wasp nest.

There are different adaptations of the structure of the cephalopharynx and the digestive tract according to the mode of feeding (Krüger, 1926). Thus in *V. bombylans* and *V. pellucens* are present: mandibular lobes, cibarial ridges and long caeca, all characteristic for saprophagous cyclorrhaphan larvae. These structures are nearly completely reduced in the larvae of *V. inanis*, and a prominent and toothed lip is present, both characteristic for predatory larvae.

The larvae of *V. inanis* are closely associated with the living wasp brood on which they feed. They are parasites and parasitoids (L3). *V. bombylans* and *V. pellucens* are less specialised in feeding habits and live mostly as scavengers in the nest of their hosts.

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Social Parasitism in Bumble Bees (Hymenoptera: Apidae): Hosts Often Succeed in Reproducing

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Species of *Psithyrus* (Hymenoptera: Apidae) are obligate social parasites of bumble bees (*Bombus* spp.). Collectively, they display a complex range of behaviours from immediate expulsion or assassination of the host queen, to prolonged cohabitation with host queens and workers. In the laboratory, host bees often reproduce in parasitized nests (van Honk et al. 1981; Fisher 1985), although this may be an artefact of laboratory conditions such as ideal food supply and enhanced worker longevity. Should host reproduction occur in the field, modifications in host behaviour to the presence of parasites could evolve, rather than immediate, irreversible escalation in which parasites are killed, or usurp all of a colony's reproductive effort. In this study, the reproductive success of host bumble bee colonies under field conditions was examined.

MATERIALS AND METHODS

A total of 10 *B. terricola* Kby nests were reared in the laboratory from queens caught in the spring of 1983 in the vicinity of Peterborough, Ontario. A *P. ashtoni* Cr. female was introduced into each of these colonies, after the first brood workers had emerged (range=4 to 6 workers). These colonies were placed in the field and observed every other day throughout the course of colony development.

RESULTS

In all parasitized domiciles host queens and parasites cohabited the comb, in some cases until the very end of colony development. Of the ten colonies, three did not survive. Of the remaining seven nests, four reared *Psithyrus* offspring ($X=22$ males, range=18-25; $X=15$ females, range= 17-25). Of these four nests, two also reared *Bombus* males (20 and 7 respectively). In one of the two parasite/host-producing colonies, host male eggs were laid prior to egg-laying by the *Psithyrus* female. The parasite began to lay eggs the last few days of June, at which time it ate any host eggs which were laid. The comb then came to consist entirely of *P. ashtoni* brood. In the other nest, host male eggs were laid at about the same time as *Psithyrus* eggs. Host males eclosed at the same time as parasite males. Host brood also was reared in the other three nests, despite the presence of the *Psithyrus* female.

The results of this study corroborate laboratory observations of *B. terricola* reproductive success in parasitized nests (Fisher 1985: only 1 of 33 colonies failed to rear at least a few reproductives). A survey of the biology of six species of *Psithyrus* which have been studied (Table I) suggests that host reproduction occurs commonly in the laboratory and field. However, in most cases investment in hosts follows or precedes investment in parasite offspring. Actual food-sharing is unlikely to be evolutionarily stable, due to the likelihood that either party could 'cheat' by eating the eggs or

Parasite	Host	Host reproductive success	Reference
<i>P. ashtoni</i>	<i>B. terricola</i> (L,F)	Yes(B,D)/No	Fisher (1985); Plath (1934)
	<i>B. affinis</i> (L,F)	Yes(D,A)/No	Fisher (1986); Plath (1934)
<i>P. bohemicus</i>	<i>B. lucorum</i> (F)	Yes(A)	Fisher (unpublished data)
<i>P. vestalis</i>	<i>B. terrestris</i> (L,F)	Yes(A)/Yes(B)	van Honk <i>et. al</i> (1981); Fisher (unpublished data)
<i>P. variabilis</i>	<i>B. pennsylvanicus</i> (F)	Yes(B)	Webb (1961)
<i>P. citrinus</i>	<i>B. vagans</i> (L)	Yes(B)	Fisher (1985)
	<i>B. impatiens</i> (L,F)	No/Yes(A)	Fisher (1985)
<i>P. campestris</i>	<i>B. pascuorum</i> (F)	Yes(A)	Fisher (unpublished data)

Table 1. Records of host reproduction in bumble bee colonies parasitized by various species of *Psithyrus*. Bracketed letters denote (following species) colonies which were either laboratory-reared and confined (L), or field/free-foraging nests (F), and (following reproductive success) host reproduction from eggs laid before (B), during (D), or after (A) comb occupation by parasites.

ejecting the larvae of the other. Indeed, coproduction of reproductives in nests parasitized by *P. ashtoni* females appears to represent a very unstable coevolutionary system in which control of proportional investment between parasite and host can very easily swing in either direction (Fisher 1986). Observations by Plath (1934) suggest that *P. ashtoni* females are better at maintaining control over reproduction in the field than in the laboratory.

Bumble bee queens may realize a modicum of reproductive success by reproducing before parasites invade their nests (or begin to reproduce), and through grandsons reared after the death or departure of the *Psithyrus* female. It is interesting to note that the species of *Bombus* least likely to reproduce in the laboratory (*B. impatiens*) is the one which is the most antagonistic towards invading parasites. The extent to which host reproduction is depressed by the presence of parasites is hard to determine, as there is such extreme variability in bumble bee reproductive success. Members of the subgenus *Ashtonipsithyrus*, for example, are not very good at suppressing males reared from worker-laid eggs (van Honk *et al.* 1981). The apparent conflict between the queen and her workers over male parentage can result in the death or expulsion of parasites which attempt to maintain a dominant egg-laying position (Fisher 1986). Observations such as these suggest that the behaviour of any Hymenopteran social parasite and its hosts should be viewed as a complex interaction between selection pressures acting on the parasite, the host queen, and host workers.

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4.6 Insect-Plant Relationship: Pollination, Coevolution of Plants and Social Insects

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Insect-Plant Relationship: Pollination, Coevolution of Plants and Social Insects

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SYMPOSIUM

Insect-Plant Relationship: Pollination, Coevolution of Plants and Social Insects

Chairperson: Hans E. Hummel

Wild Bee Community in an Agricultural Area of Rio Grande do Sul, Southern Brazil, and its Impact on Pollination of Beans and Sunflowers

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A great number of studies on the importance of *Apis* as a pollinator of crops has been published to date (e.g. Free, 1970). However, little attention has been paid to the function of other social bees, especially the tropical Meliponini and solitary bee species. Since the introduction of *Apis* to the American Tropics, *Apis* has played a dominant role in crop pollination. However, wild bee communities are important for crop plants which are rarely visited by *Apis*. As pollinators of endemic non crop plants, wild bees are important factors for plant species conservation and maintenance of wild flower gene pools.

MATERIALS AND METHODS

From August 1984 to July 1985 wild bees were sampled in 2 week intervals on wild flowers and in sunflower and bean plantations of the Estacao Experimental Fitotecnica de Aguas Belas, Rio Grande do Sul, Brazil. The effect of bee pollination on seed set in sunflowers and beans was determined by comparing nylon covered and exposed parts of the plantations.

RESULTS

In total, 646 bees were sampled which are distributed among 74 species in 32 genera of 6 families. Of the Apidae 5 species were recorded which represent 43.6% of the individuals and thus are the most abundant bee family. The most frequent species is *Plebeia* (Mourella) *caerulea*, which makes up 14% of the specimens. Species richness is highest in the Anthophoridae and Halictidae, which together represent about two thirds of the species.

Out of the 6 bee families the Apidae, Anthophoridae and Halictidae were present throughout the year. The Andrenidae are active from winter till the end of summer and the Megachilidae from spring to the beginning of autumn. The Colletidae were restricted to a short period during spring and summer.

Of melittophilous plants, 69 species in 28 families occur at the study site. In this plant community the Compositae dominate with 23 species followed by the Solanaceae with 6 and the Leguminosae with 4 species. The most abundant species is *Sida rhombifolia* (Malvaceae).

The 5 species of Apidae visited a total of 43 plant species. *P.* (Mourella) *caerulea* was the most generalistic species which visited 19 plant species with preference for *Sida rhombifolia*. The 25 species of Anthophoridae visited 29 plant species, the 24 halictid species visited 39. Megachilidae and Andrenidae were found on 10 and 12 plant species respectively. The species of the other bee families visited between 1 and 10 plant species. 3 bee species were specialists which visited 4 or fewer plant species.

During the flowering season of beans and sunflowers 64 bee species

in 29 genera were present in the habitat. 11 species visited beans, 15 were recorded on sunflowers. The most abundant species on beans were Bombus atratus, Megachile auriventris and Thygater analis. All other species (including Apis) which were found on beans were not able to enter the flowers. On sunflowers the most abundant bees were Bombus atratus, Augochlora amphitrite and Megachile angularis.

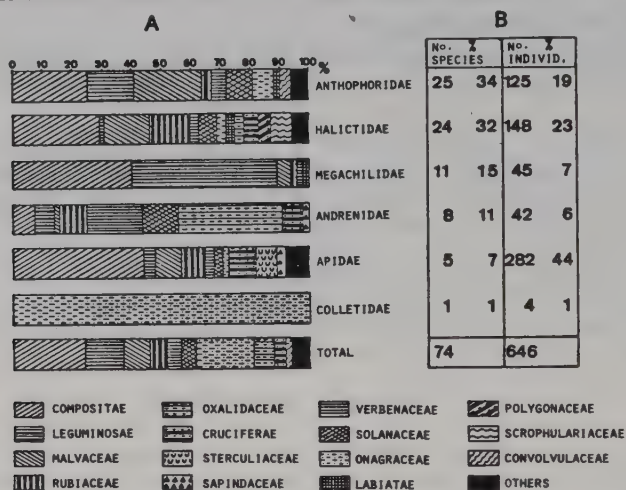


FIG. 1: A. RELATIVE ABUNDANCE OF 6 WILD BEE FAMILIES ON 28 PLANTS FAMILIES AND B. NUMBER AND RELATIVE ABUNDANCE OF SPECIES AND INDIVIDUALS OF 6 BEE FAMILIES.

Seed set in beans was not significantly augmented by bee pollination (+16%). In sunflowers a 52% increase was recorded due to pollination. Most effective were megachilid bees which pollinate high numbers of florets during extensive walks on single sunflower heads.

DISCUSSION

The rank of species richness of all families recorded at Aguas Belas showed no differences in comparison to other bee communities studied in Southern Brazil (Sakagami & Laroca, 1971; Laroca et al., 1982; Knoll, 1985). The number of bee species captured was lower than in most other faunistic studies done in northern parts of Southern Brazil, which reflects a diminishing species abundance on a north-south gradient towards the subtropics.

The bee-flower relations demonstrate that the 5 species of Apidae are the most generalistic ones in their use of pollen and nectar plants. Therefore, anthropogenic changes, especially in agricultural areas, will primarily affect bee populations of the non-Apidae which make up more than 90% of the species in the habitat studied. Although the impact of wild bee communities on crop pollination may in general not reach an economical threshold, they have to be considered as additional pollinators of many crops and may in some cases outrange the importance of Apis, as it is the case of sunflowers, where the megachilid bees are more effective due to their different mode of pollen collecting.

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On the Pollen Harvest by Africanized *Apis mellifera* and *Trigona (Trigona) spinipes* in Sao Paulo

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Trigona spinipes has one of the most populous colonies among Meliponinae and has temperature uniformity within nests, approaching that of *Apis* (Sakagami, 1982). The communication system of food sources is probably as efficient as in *A. mellifera* (Kerr et al., 1981), and foragers exhibit monopolistic and aggressive behavior during foraging.

A. mellifera and *T. spinipes* are the Apidae of greatest relative abundance and the most generalistic plant visitors among the Apidae in the gardens of IBUSP (Knoll, 1985; Cortopassi-Laurino, 1982). Here we make a colonial approach of utilization of floral resources. We hypothesize that polylecty and sobreposition at sources should be high, but differential utilization of each source could be achieved by both species, considering their colonial necessities and foraging strategies.

MATERIAL AND METHODS

During one year (Aug/80-Aug/81) monthly samples of pollen were taken from one colony of *A. mellifera* (**Am**) and one colony of *T. spinipes* (**Ts**), both placed in the gardens of IBUSP. Pollen slides were prepared and frequency of pollen types in samples were estimated. These frequencies were used to calculate some ecological parameters, such as niche width, evenness and percentage similarity (Kleinert-Giovannini et al., 1986).

RESULTS AND DISCUSSION

The high degree of polylecty of **Am** and **Ts** (Fig. 1A) probably is related with the large size of colony population (aprox. 50.000), as suggested by Sommeijer et al. (1983), the intensity of foraging throughout the year (less susceptibility of colony growth to abiotic factors), and to home range. On the basis of the size of foragers of **Ts** (7 mm), home range of colonies (Wille, 1983) should encompass all the gardens, where 242 species belonging to 73 botanical families were available. The same could be applied to **Am** (Free, 1970).

The families with the greatest number of species in samples were the same for both species (Fig. 1A), but **Am** visited Compositae more than **Ts**. These families are also well represented in the gardens. So, diversification of pollen harvest seems to be not selective and approaches distribution by chance of foragers in field.

There was high frequency of pollen harvest in some families with just a few species in the gardens (Fig. 1B), but with a good surplus (large number of individuals or productive sources). Liliaceae and Palmae were exploited mainly by **Ts**, while **Am** visited Leguminosae and Moraceae. The monthly percentage similarity (PS) ranges from 0.05 to 0.84, and 0.27 on average. Similarity was high in pollen harvest from *Eucalyptus* spp (Myrtaceae), but some sources were little or not shared. For instance, *Archontophoenix* sp (Palmae) was visited by **Ts**, while some Le-

guminosae (*Caesalpinia pelthophoroides*, *Mimosa velloziana* and *Tipuana speciosa*) were visited mainly by *Am*. It seems that *Ts* and *Am* show differential preferences for some plant taxa.

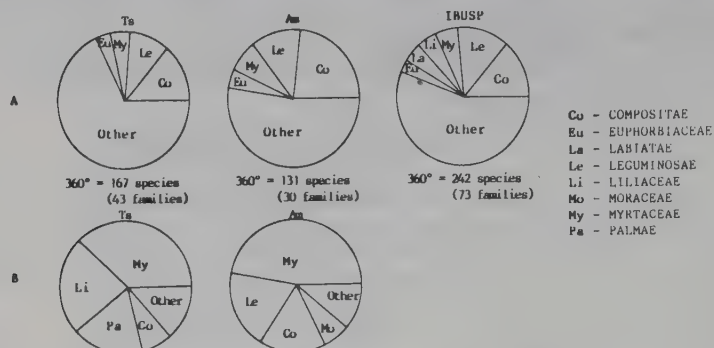


FIGURE 1 - Pie diagram of the most represented families in pollen samples of *T. spinipes* (*Ts*) and *A. mellifera* (*Am*), and in the garden of IBUSP. A- in number of species; B- in frequency.

The values of niche width (H') for monthly samples of *Ts* range from 0.89 to 2.55, and on average 1.62, and for *Am* from 0.83 to 2.55, and on average 1.62. The average evenness (J') for *Ts* and *Am* were 0.46 and 0.47, respectively. In general, there was a high monthly diversification of pollen harvest, but just a few sources were intensively exploited. Just 12 and 08 pollen types with representativity higher than 10% were found in samples of *Ts* and *Am*, respectively, during a year. These data reveal some specificity of food gathering, probably related to preferences for few productive sources (Eickwort & Ginsberg, 1980;) such as *Eucalyptus* spp and *Archontophoenix* sp.

Many pollen sources are found by foragers from both species and harvest is similar by chance, but few sources are intensively exploited by colonies or shared between species. These facts suggest either floral preferences and that foraging strategies and colonies needs may reduce simultaneous use of some sources.

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Mating and Pollinating Behavior of *Callonychium* sp. (Andrenidae) on *Petunia integrifolia* and *ovalifolia* (Solanaceae)

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Close relations between female bees and their food plants provide ample possibilities for the evolution of male mating behavior in connection with female foraging behavior. Here we report on the mating and pollinating behavior of *Callonychium* sp. bees on flowers of *Petunia* spp.

MATERIALS AND METHODS

This study was conducted from November 1985 until February 1986 at three study sites in Rio Grande do Sul, Southern Brazil. At each site records were made from 9.00h to 17.00h on the activities of individually marked *Callonychium* sp. bees in patches of flowering *Petunia integrifolia* and *P. ovalifolia*. To check for visits of *Callonychium* bees on other flowers than *Petunia*, extensive bee collecting and observations were also carried out in the surroundings of *Petunia* patches.

RESULTS

Callonychium females forage almost exclusively on *Petunia* spp. Thus they are oligolectic with a strong tendency to monolecty. Males of *Callonychium* were only found in *Petunia* patches where they search for females. Three search strategies could be distinguished:

1. Patrolling. Patches of *Petunia* may be patrolled by one or up to hundreds of males. Males interrupt their flights by short touchdowns on flowers or by random visits in or on the flowers. In flight, males show no antagonistic behavior towards each other. Patrol flights rarely last longer than 60 sec. but are carried out frequently so that about 60% of male movements in a patch is patrolling.
2. Inspecting. Interruptions of patrol flights at flowers may last up to 10 min. Males either stay on the petals or enter the corolla, where they remain motionless at its opening. Females landing on the flower are quickly approached and mounted. Conspecific males landing on an occupied flower are attacked. About 20% of male movements in a patch end with flower visits and female expecting behavior.
3. Perching. Males interrupt patrol flights to rest and wait for females on perches, which are mainly stones adjacent to *Petunia* flowers. Females approaching a flower near the perch are grasped when landing on it. Males do not chase females in flight. When male and female abundance in a patch is high, males increase the relative number and duration of patrol flights. Thus their search strategies undergo seasonal changes.

Females of *Callonychium* are polyandrous and mate several times per day. Copulation is always initiated in or on flowers of *Petunia*, where it may last a minute or more. The couple then flies off *in copula* to visit other flowers, where the female collects pollen. They finally leave to land outside the patch, where copulation continues for more than 15 min. They then take off separately.

Bee species other than Callonychium are extremely rare on blooming Petunia ovalifolia and integrifolia (1-2 individuals/day). Analysis of pollen loads reveals that Callonychium females are flower constant on Petunia.

When Callonychium males leave a Petunia flower, they turn from a backward to a forward position over the anthers. Thereby they passively take up pollen with long ventral setae. As they visit various flowers of different Petunia individuals and frequently switch between patches, males can be considered to be passive additional pollinators of Petunia spp.

DISCUSSION

In this association of Petunia and Callonychium, both sexes of the bee are the main pollinators of the plant, whereas the flowers provide the bees with nectar and pollen and serve as the only site, where males and females of Callonychium encounter and where copulation is initiated.

In Callonychium the males searching behavior for mates is adapted to the oligolectic foraging behavior of the females. The predictability of females at small areas results in high abundance of conspecific males in Petunia patches. As a consequence nonaggressive patrolling of the flowers, rather than establishing territories may have been favoured by sexual selection. The frequencies with which the three strategies are applied by a male indicate that the chances to meet a female are different for each strategy: patrolling the flowers may be more effective than waiting or perching.

Flights in copula can be observed in many bee species with polyandrous females (Alcock et al., 1977). In those cases where sperm precedence occurs or can be assumed, these flights are explained as male strategy to protect their genetic investment and take advantage of being the last male to mate with the female before oviposition (Eickwort and Ginsberg, 1980).

In the case of Callonychium apparently no sperm is transferred during flights in copula. It is probable that insemination only occurs when the couple has left the flower patch to copulate in a resting position. After these prolonged copulations the sexes leave separately. By flying in copula Callonychium males thus may not guard their genetic investment, but protect their potential mates from takeovers until the couple has left the area of high competitor abundance. By accompanying a foraging female before mating, males may reduce the probability that their mates return to a flower patch after mating. For Callonychium males guarding a female after insemination may thus not be advantageous, as the female has already foraged in Petunia patches and will probably not encounter other males on her way back to the nest.

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Role and Nature of Plant Chemicals Implied in Entomophilous Pollination: Sunflower-Honey Bee Model

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Among pollinating insects, honey bees have a particular economical interest for crop improvement through hybrid seed production. In sunflower, which is now the 2nd oilseed crop in the world, hybrid seed production strictly depends on entomophilous pollen carrying from male to female lines. In order to analyze sunflower-honey bee relationships, basic studies of foraging behaviour and of specific stimuli releasing this behaviour have been set up. Such studies imply pluridisciplinary approaches (behavioural and chemical analyses) carried out at different levels (field and laboratory experiments).

Foraging behaviour is based on a conditioning process where plant chemicals are mainly involved (MASSON, 1982) : while foraging worker bees use to associate food uptake (nectar, pollen) with plant aromas, which are then memorized as orientation cues. Therefore, we tried to determine how far honeybees are able to discriminate among sunflower genotypes, using scents associated to food reinforcement. This should lead to define molecular criterions of plant attractiveness, likely to become tools for plant improvement through entomophilous pollination.

Behavioural data from field experiments :

Experiments were carried out under tunnels with honey bee colonies pollinating two couples of parental lines of commercial hybrids (Marianne and Mirasol) producing different hybrid seed yields (low yield for parents of Marianne and high yield for parents of Mirasol). It appeared that foragers exhibit a selective behaviour towards parental lines of Marianne (with a great preference for the female line) while they visit randomly parents of Mirasol. Such behaviour pointed out foragers ability to discriminate among different plant genotypes and was directly related to the differences of seed yields.

Chemical factors of discrimination :

- Gustatory cues : honey bees use to visit sunflower mainly for nectar collection. We set up studies of nectars amounts and total amounts of sugars in nectar, usually considered as the main criterions in bees preferences. But quality of nectar sugars and their relative proportions were also taken into account. Nectar samples were collected from the four lines and then analyzed by gas chromatography technic which allowed to estimate proportions of each sugar of the nectar.

For the genotypes considered, total amounts of nectar or total amounts of sugars in the nectars could not be related to foragers preferences. But a "glucidic pattern" of nectar appeared to characterized each genotype : fructose, α and β glucose and sucrose were identified with proportions proper to each genotype, along several years and different places.

Moreover, it appeared that sucrose was mainly involved into bees choices : thus, preference for the female line of Marianne was related

to high amounts of sucrose, while the male line, equally supplied in fructose and glucose, had nearly no sucrose. When both parental lines had a lack of sucrose (Mirasol case), honeybees did not significantly discriminate among the two lines.

- Olfactory cues : a technic of headspace trapping applied to living sunflower heads was set up, leading to a qualitative extraction, very close to natural plant emissions. Among hundreds of compounds detected by gas chromatography, statistical comparison of chromatographic profiles showed significant inter-genotypes differences for 7 compounds among 130 for Marianne parents, and for 20 compounds among 250 for Mirasol parents. Thus, among Marianne parental lines, honey bees are able to discriminate through a few compounds among complex chemical blends.

Basic mechanisms of olfactory discrimination among complex blends :

In order to define in a more analytical way the role of the chemicals involved in foraging behaviour, the foraging situation was simulated in controlled conditions, with a bioassay using an artificial flower device. The bioassay was based on an associative conditioning where a sugary reward (= unconditioned stimulus) mimicking the nectar was associated with an olfactory signal (conditioned stimulus) constituted by a floral aroma ; this conditioning was applied to a whole colony flying freely in a flight room. After a conditioning period where foragers memorized the scent associated with the food source, a choice was given between the conditioning scent and a control or another scent, without any reward. Then foragers distribution allowed to appreciate their ability to recognize the conditioning scent or to discriminate among the different scents.

A typical sunflower aroma from a blend of genotypes was obtained by dichloromethane extraction and molecular distillation under high vacuum conditions. Chemicals were then identified by coupled gas chromatography and mass spectrometry ; among hundreds of compounds detected, 84 have been indexed and 58 were identified (ETIEVANT *et al.*, 1984). The whole aroma was chemically fractionned ; fractions were successively presented to honeybees, compared to the global aroma. Behavioural responses showed that a fraction limited to about 20 polar compounds allowed the recognition of the whole aroma (PHAM-DELEGUE *et al.*, 1986). Thus, honey bees were able to identify a complex volatile blend from a restricted active fraction.

Currently, in order to precise the biological activity of volatile compounds, a method of coupling simultaneously GC with animal detection (electrophysiological and behavioural recordings) has been set up and should be an useful tool to elucidate basic mechanisms of insect responses to allelochemicals.

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Role of Pollenkitt in Pollen Host-Plant Recognition by Male and Female Solitary Bees

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Host-plant specificity exhibited by many solitary bee species for pollen (while generalist for nectar) suggests that pollen cues are involved in initial flower selection by newly emerged bees (Linsley, 1958, Thorp, 1969). Foraging honey bees use pollen odor in orientation (von Frisch, 1923, von Aufsess, 1960), and they are attracted to the surface oils - pollenkitt (Louveaux, 1959, Lepage and Boch, 1968, Hohmann, 1970), which contain a diversity of lipids in species-specific mixtures (Dobson, 1985). To determine the possible role of volatile pollenkitt chemicals in pollen host-plant recognition by both male and female solitary bees, preference tests were performed on a population of *Colletes fulgidus longiplumosus* Stephen which visits mainly Compositae and collects pollen from *Grindelia stricta* (Dobson, 1984, 1985).

MATERIAL AND METHODS

Responses to different plant "odors" were tested on foraging inexperienced (23 ♀ and 55 ♂ emerged in the laboratory) and foraging-experienced (24 ♀ and 20 ♂ collected in flight) bees for one season. Preferences were evaluated by the number of feeding responses (landing on samples with proboscis extension) elicited in bees when offered a choice of four plant species in the form of flowers, whole pollen, or pollenkitt, each sample covered with cheesecloth or wire screen (feeding excluded).

RESULTS AND DISCUSSION

Inexperienced bees showed a significant preference ($p < .001$, Wilcoxon Rank-Sum Test) for their host plant when presented odors of flowers, whole pollen, and pollenkitt (Tab. 1), supporting preliminary studies

Tab. 1. Percentage of total feeding responses by female and male inexperienced bees to different odor samples, and percentages of bees observed to probe on any sample from each species

Species (family)	Flowers		Pollen		Pollenkitt		% bees	
	♀	♂	♀	♂	♀	♂	♀	♂
<i>Grindelia stricta</i> (Compositae)	53	56	55	61	45	62	100	91
<i>Eriophyllum staechadifolium</i> (Compositae)	24	14	20	23	36	20	74	58
<i>Erigeron glaucus</i> (Compositae)	0	6	20	13	12	9	65	34
<i>Lupinus arboreus</i> (Leguminosae)	23	24	4	5	7	9	52	49
Total no. responses	87	217	93	127	73	127		
Total no. bee-test-trials	108	198	96	156	88	134		

(Dobson, 1984). Olfactory-based host-plant preferences are thus preformed when bees emerge from the nest. In addition, the number of bees noted to respond was higher on Grindelia than on other species (Tab. 1).

Preference patterns, and overall response levels (average number of probings per bee-trial), were similar for females and males (Tab. 1). However, a larger percentage of females responded on each species than did males (Tab. 1). This was generally most pronounced in tests using whole pollen or pollenkitt, suggesting that females (the pollen collecting sex) may have a greater sensitivity to volatile pollen chemicals.

Proportions of feeding responses directed to flowers, on the one hand, and whole pollen or pollenkitt, on the other, indicate that attractant or deterrent chemicals are present in both parts for some flower species (Grindelia, Eriophyllum), but not for others (Erigeron, Lupinus). Furthermore, the similar response levels received by whole pollen and pollenkitt demonstrate that volatile pollen cues are indeed located in the pollenkitt.

Field-experienced bees showed a low feeding response to odors of flowers and pollen. During foraging (learning), the bees' olfactory discrimination between food and non-food species appears to become more fine-tuned while reliance in host-plant orientation on olfactory signals, particularly from pollen, decreases.

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Evolutionary Ecology of Some Ant-Plant Mutualisms

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Much recent research into ant-plant mutualisms has demonstrated that the outcome of interactions is regulated by ecological variables. These may be numerous, interactive and often highly localized, for example, the distribution and abundance of reward-bearing plants, ant nests, and non-mutualists that remove rewards parasitically. This highly dynamic situation means that at any given time and place some or all of the interacting species may experience full, intermediate or episodic benefits, or no benefits at all. The cohesive evolutionary force amid this variability is the demonstrable selective advantage experienced by interacting species when all variables are conducive to the effective functioning of the mutualism. Thus, it is no surprise that some field studies show no benefits from apparent mutualisms, while others show significant increases in fitness resulting from similar mutualistic interactions. A discussion of examples of seed dispersal by ants and ant-guard systems will be followed by a consideration of the role of coevolution in these interactions.

4.7 Economic Aspects

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SYMPOSIUM

Economic Aspects

Chairperson: Abraham A. Mabelis

Pest Status of Crazy Ant *Anoplolepis longipes*(Jerdon) in Karnataka, India, and Causes for its Outbreak

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There was no mention of an economic importance of crazy ant *Anoplolepis longipes* until when Soans and Soans (1971b) reported its association with *Aphis craccivora* from India. In recent years it has assumed a pest status in about 2000 hectares, covering ten villages in Maddur Taluk, 120 km from Bangalore, Karnataka. The causes for its outbreak and remedies have been investigated.

MATERIALS AND METHODS

The survey was conducted in the ant infested villages for cropping patterns, irrigation systems, methods of organic waste disposal, topography and vegetation of the land. Important plant species and their sucking pests in the locality, breeding behaviour of the ant and its distribution were documented. Five insecticides against the ant were tested under field conditions.

RESULTS

The survey conducted in the ant infested villages revealed that *A. longipes* did not exist in that locality a decade prior to 1980. It was suspected to have been accidentally introduced to Karkalli village during the 1970s along with the firewood logs brought from the forest for tobacco curing (Veeresh and Gubbaiah, 1984). The newly introduced ant found the locality very ideal, both weatherwise and foodwise. The temperature ranged between 22°C-35°C with an annual precipitation around 700mm, distributed over 7 to 8 months, in addition to channel irrigation available round the year. The major crops grown in that area are sugarcane, mulberry (*Morus alba*), and coconut. Scattered among these were nearly 28 major plant species.

Initially, the ants multiplied under rotting coconut fronds and leaves, as well as under manure heaps frequently found in the area. Ants spread to the field and on to the neighbouring villages along with the organic manure carried. Thus, in about ten years, they occupied an area of over 2000 ha, covering 10 villages.

During the course of its establishment in the locality, dominant insects like *Oecophylla smaragdina* and *Leptogenys processionalis* were eliminated. The crazy ant became a major predator of termites in place of *L. processionalis*. Soans and Soans (1971a) have reported intergeneric competition and replacement of other ants by *A. longipes*.

In dwellings, they ran everywhere in large numbers, irritating inmates, sleeping babies, running even over the food served to eat. A similar nuisance has been reported from Scychellis by Haines and Haines (1978). Over 500 ha of mulberry gardens infested with the ant were severely affected by powdery mildew (*Phyloctinia corylea*) making the leaves unfit for silkworm feeding. In the same locality, mulberry grown under rainfed condition was free from both ants and powdery mildew,

suggesting that the ants spread powdery mildew and also preferred a moist place to establish.

In sugarcane fields the ants excavated the soil at the base and loosened the roots, causing the plants to dislodge with slight wind. Irrigation became difficult due to deep holes made by them. The mealybug (*Saccaricoccus sacchari*) greatly increased in number and reduced the sugarcane yield drastically.

Nearly 375 plants/trees, comprising 28 species, were examined in the locality and almost all had one or the other sucking insect (Scales, mealybugs, aphids) encouraged by the ants. Trees like *Cassia tora* had no sucking insects, yet the ants were plenty on them, perhaps for the presence of plant exudates. Large colonies of ants, however were found at the base of a few species of trees, particularly *Acacia arabica*, with the underground chamber going up to a one meter down.

Normally breeding took place either under the rotting debris of coconut leaves and fronds, or under manure pits or stones. The active breeding period was between June-September with the onset of South-West Monsoon, when large numbers of eggs, young ones and pupae were found, often in stratified layers. Alate females and males, and foragers were found in various proportions. One of the convenient methods of collecting foragers for population counts was to spread a cloth, remove it every 20-30 seconds and shake the contents into a container.

According to Rao (personal communication) who conducted migration and dispersal studies using the radio isotope ^{32}P , the foragers are normally confined to 6 - 8 m radius from their nest. Dispersal was mainly through the alate females or aided by man.

Five insecticides including Chlorpyrifos 20EC at 0.25 %, Aldrex 30EC, 0.5 %, BHC 10 % dust, Heptachlor 6 % dust and Parathion 2 % dust at 25 kg/ha were applied in four replications at random to the base of trees and breeding grounds. Although initial kill was obtained in all treatments, maximum protection for over six months was obtained by Heptachlor treatment.

In conclusion, it may be stated that availability of plants like sugarcane and *Acacia arabica*, on which foragers found plenty of food, rotting debris for breeding and the presence of moisture round the year were found to be favourable factors for the outbreak of ants. The ants caused nuisance, made irrigation wasteful and loosened the roots of crops. Periodical application of soil insecticides at vulnerable places reduced the population to non-pest level.

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How to Protect Ants in an Over-Developed Country?

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ENDANGERED SPECIES

Effective conservation depends on sufficient information on trends in ecosystems and on an understanding of their causal factors. Therefore comprehensive and accessible data bases are needed. Such data bases are internationally important for informing and influencing governments in their conservation policies.

Data about the status of insects are scarce. For evaluating and predicting situations, we need to know characteristics of threatened species, as well as about changes in their environment. The Ant Specialist Group of the Species Survival Commission (IUCN) is one of the groups of specialists that share this task.

One task is to collect and summarize data on vulnerable and endangered ant species. Compilations of the status of the species in each region or country are needed for evaluating the status of as many ant species as possible. Unfortunately this can only be done for regions where ant distributions are known.

More regional data on distributions are required for knowing the status of species within their total range. For evaluating regional data, the IUCN categories of species being threatened can be used, only applying to local situations. For example, the categories of being threatened, mentioned in the List I made of endangered ant species in the Netherlands, apply only to the Dutch situation. Nevertheless, the way in which the species is threatened in part of its distribution range may affect its future status when the causal factors continue to operate.

FACTORS OF THREAT

About half of the Dutch ant species are either vulnerable, endangered, rare or extinct. Four of them (*Camponotus ligniperda*, *Polyergus rufescens*, *Formica truncorum*, and *Leptothorax unifasciatus*) occur at their range margin, and were therefore probably never common, whereas the distribution of five other species is insufficiently known. Four of the endangered species in the List, are permanent social parasites and consequently both rare and locally distributed. They may seem to exist close to the edge of extinction, whereas in fact they may be not endangered at all. The remaining species (i.e. 36 %) have declined, particularly because of human activities, as air pollution and modern agricultural practice.

1. SO_2 , NO and NH_3 attribute to the acidification of the Netherlands for 50 %, 20 % and 30 %, respectively. Electric power stations, oil refineries and other factories are the main producers of SO_2 , while NO is not only produced by electric power stations and factories, but also by traffic. NH_3 comes mainly from animal dung (ca. 90 %).

Once deposited, it will be converted into HNO_3 by bacterial activity. Cattle-breeding is thus responsible for about $\frac{1}{3}$ of soil and water acidification.

Moreover, it is responsible for soil nitrification. At present, through rainfall nature reserves receive on average 46 kg of nitrogen per hectare annually, which is more than the farmers used at the beginning of this century. Increased soil acidification and fertilization are particularly detrimental for ecosystems of poor soils, such as heathland.

Acid rain make *Calluna*-heather more vulnerable to frost damage, leading to its dying off on an extraordinary large scale after the last winter. Moreover, nitrogen rich heather plants are more favourable for the development of the heather beetle (*Lochmaea suturalis*) and this led to more frequent outbreaks. As a result, *Calluna*-heather died over extensive areas and grasses took over its dominance. Consequently, nests of heathland species, such as *Formica cunicularia*, *F. rufibarbis*, *Myrmica sulcinodis* and *Tapinoma erraticum*, declined.

2. Modern agricultural land-use is highly detrimental for many species. At present most Dutch farmers have more animals than their land can support: too many animals relative to grassproduction and too many for using all the manure. This manure dumped on their land leads to soil and groundwater pollution with nitrogen (N), phosphorus (P), potassium (K), and heavy metals (Cu, Zn, Cd). Particularly the nitrate load of the soil is a serious Dutch environmental problem. It has not only a negative effect on diversity and stability of ecosystems, but it also affects public health, the health of the cattle, soil productivity and the quality of agricultural products. In this way the natural environment is destroyed by short-term solutions for the problem of excess manure. Consequently, present agricultural grassland is extremely poor in species; even the most common and eurytopic ant species, *Lasius niger*, cannot survive in such overmanured environments. The decline of *Lasius flavus*, still very common in Dutch grasslands a few decades ago, and now restricted to nature reserves, also illustrates the negative effect of this practice.

CONCLUSIONS

Legal measures for protecting species are of little use when soil and air pollution as the main causes for decline, are not tackled seriously. Technological measures may help diminishing pollution, but can not solve the problem of increasing environmental deterioration. The solution of this problem depends on the willingness of people to optimize rather than maximize production. Affluent societies are sufficiently rich to make amends on production for preserving natural resources for posterity, that in the absence of prohibition or regulation, would continue to be spoiled and to be squandered.

Therefore, management of human populations and activities should be promoted to maintain a sustainable balance between natural resources and a modest standard of living above the minimum survival level for all of mankind.

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Colony Structure of *Liometopum apiculatum* M. and *Liometopum occidentale* var. *luctuosum* W.

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INTRODUCTION

Ants of the genus *Liometopum* are edible in different places of Mexico; for this reason, they are known as "escamoles". Most of all are eaten the immature stages of the reproductive caste (Conconi et al., 1982a; Conconi et al., 1982b) and also those of the worker caste and reproductive adults that have a high nutritive value (Conconi et al., 1984). We studied their colony structures because of their nutritional and economical importance.

MATERIAL AND METHODS

The field work was made in Tulancingo (Hidalgo State), an arid region, for the first species and in Tlalpujahua (Michoacan State), a temperate region, for the second species, in order to observe the nuptial flight, and for collecting settler queens. Afterwards, we followed the life cycle at the Entomological laboratory of the Institute of Biology UNAM. We determined also their kind of nutrition (Conconi, 1983), the extension of their trophic fields using I 131 (Conconi, 1986), the number of nests per area, and, making excavations, their nest structure (Conconi, 1986).

RESULTS

In *L. apiculatum* M., colony foundation is made by haplometrosis, and in *L. occidentale* var. *luctuosum* by pleometrosis of 2 to 40 queens. Generally *L. apiculatum* lives in arid and semiarid regions, and *L. occidentale* in temperate zones. Sometimes, they live sympatrically, and always at an altitude of 2000 m in pending ground. Their nest structures are similar. The queen is well protected at a remote place about 6-8 m from the big "trabecula", where the "escamoles" are deposited and exploited. The "trabeculas" are connected by various galleries. In *L. apiculatum*, we found 3 or 4 big, polycalical trabeculas. The diameter of the trabeculas is thin in *L. apiculatum* and thick in *L. occidentale*. Both species are omnivorous, having their trails at the surface for *L. apiculatum* and as tunnels under the surface for *L. occidentale*. The life cycle is shorter in *L. apiculatum* and their productivity is greater. The number of nests per area is also higher (6), the extension of their trophic fields is smaller, and the trail and alarm pheromones are used in higher concentrations than in *L. occidentale*. Their polymorphism is bimodal with diphasic allometry.

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Damage of *Coptotermes ceylonicus* Holmgren to Structural Wood in Telengana Region of Andhra Pradesh

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Among the subterranean termites, species belonging to the genus *Coptotermes* are most serious pests of structural-wood. The present paper deals with the wood-destroying activity of *Coptotermes ceylonicus* in different types of human dwellings such as Rod-Cement-Concrete (RCC), Tile-Roofed (TR) houses, and Huts (H) in the South Indian city of Warangal and its surrounding villages in Andhra Pradesh.

MATERIALS AND METHODS

Surveys were conducted during December 1984 - March 1986 on termites damaging different types of structural wood in the RCC and TR houses, and in the H in Warangal city and its surrounding villages in Telengana, the semi-arid region in A.P. The house owners were enquired regarding the termite damage in their houses. The indoor wood-works in the houses were inspected for signs of termite activity and damages were examined rending and exposing the interior portion by a chisel. The intensities of damage were quantified according to a rating based on a 0-5 scale (Beal, 1979).

RESULTS

The surveys revealed that 57.7% of the houses in the city and 77.2% of the houses in the surrounding villages were damaged by different species of termites, including *C. ceylonicus* damaging 8.2% of houses in the former and 3.4% of houses in the latter environments.

The damage of *C. ceylonicus* in different localities of the city and villages, in relation to soil-types such as red and black soils as well as vegetation (as these factors directly influence the distribution of termites), indicated that the termite infestation to structural-wood was mostly confined to the red-soil localities of the city and the villages, except a RCC house surrounded by trees which was damaged in the city's black-soil locality. In the red-soil localities, the termites damaged the RCC houses where such houses were more scattered as compared to localities where the houses were congested with a negligible number of trees in the vicinity. However, more houses, particularly the TR ones, were damaged in localities congested with houses both in the city as well as in the villages. A single TR house was damaged in the locality of the city with scattered houses but with more trees in the immediate surrounding, whereas two TR houses were damaged in the villages with less trees in the vicinity.

Of the three different types of houses, the TR houses were maximum damaged by *C. ceylonicus*, followed by the RCC houses and the H. It is probably because of the presence of a greater number of TR houses as compared to the RCC ones. Table 1 depicts the intensities of damage caused by *C. ceylonicus* to structural-wood both in the city and in the villages. In addition to the woodworks damaged in the RCC houses in the

city, different types of books, files and papers were also damaged, which may be due to the lack of much wood-food in these houses as compared to the TR ones. Serious damage to the books, papers and the cloth coverings was recorded in an office building in its ground floor with long exploratory runways on its cemented walls. Besides, surprisingly the books, files and papers kept in an iron shelf with closed doors in another office building were damaged where the termites entered into the shelf through a small perforation in one of it's legs. In the TR

Table 1. Intensities of damage to different types of indoor woodworks and books, by *C. ceylonicus* (based on Beal, 1979).

Articles	City			Villages		
	RCC	TR	H	RCC	TR	H
Woodworks						
Door	5	5	0	0	0	0
Window sash	5	0	0	0	0	0
Rafter	0	5	0	0	5	0
Joist	0	5	0	0	5	0
Eaves	0	3	2	0	3	0
Books and Papers						
Register	5	0	0	0	0	0
Files and Papers	5	0	0	0	0	0
Cloth coverings	4	0	0	0	0	0

office buildings, where the termite damage was given little attention, even the door panels and the window sashes were damaged. In the private TR houses, however, only the woodworks such as joists, rafters and eaves which were unreachable, were damaged. The eaves of the H in the city were damaged, too. Among the woodworks, the proximal portions of the vertical and the upper horizontal axes of the door-panels in the RCC houses, and the rafters and joists in the TR houses were maximum damaged.

An estimation of the present-day cost in rupees (Rs) of the damage caused by *C. ceylonicus*/house in the city and in the villages, reveals that about Rs. 3000/- to 4000/- were spent for the repair and replacement of the damaged wood. It was because of characteristic damage of *Coptotermes* destroying the inner sound wood.

One of the authors, Mr. Ch. Sammaiah is thankful to UGC (New Delhi) for providing a teacher-fellowship to him.

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Termites as an Economic Factor

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Termites play an important role in destroying wood-based constructions especially in tropical and subtropical regions. Every year the destruction of man-utilized wood by termites comes to several million dollars. Only one genera has become adapted to the Northern temperate zone.

Termites are not related to ants but to cockroaches. They live in social communities consisting of different castes. Every caste has another function. The development of larvae into different castes is influenced by pheromones.

Termites have to be differentiated in two groups in relation to their way of life: subterranean termites and drywood-termites. Subterranean termites require permanent moisture whereas drywood-termites are able to exist without moisture. In case of termite attack the outer layers of timber remain intact and for that reason in most of all cases the attack is discovered when the damage is endangering the static.

The destruction of houses in Hamburg by *Reticulitermes flavipes* in the mid 50 s is the wellknown attack of termites in the temperate zone. The area confounded was about 55 ha. The distribution of termites took place by supplementary reproductives.

Protecting buildings from termites has to be differentiated in the protection of new buildings and the protection of existing buildings. It is also important to distinguish between protection against subterranean termites with chemicals and termite-shields and the protection against drywood-termites with durable timber. Naturally resistant timber contains chemicals which are repellent or toxic for termites. But resistant timber is rare and expensive so that the use of chemicals is necessary to make timbers durable.

By using different methods of termite-control it is important to realize what kind of pest it is: is it a drywood-termite or a subterranean one. Then it is possible to use one of the methods like physical treatments with hot air or fumigation, biological fighting with useful insects or the pest itself, biotechnical methods using attractants, repellents, biogenetic insecticides, pheromones and hormones. But fighting with natural means also has its problems.

Successful were attempts with fungi. Fighting with fungi against termites has two aspects: fungi can kill termites by producing toxic digestion products or they attack termites' tissue. Success is also expected by the application of hormonmimetica. They are able to mislead the hormonal steering-system of termites.

Feeding and Survival of Subterranean Termites on Boron Treated Wood

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The objective was to determine the percentage boric acid equivalent that must be retained in pine (*Pinus* spp.) wood to protect it from two subterranean termites (*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki). Wood was pressure treated with various concentrations of disodium octaborate tetrahydrate (TIM-BOR). Random samples of wood treated at each concentration were exposed to termites in no-choice and choice laboratory tests. Other samples were chosen for boric acid analysis, spraying with a color reagent, or exposure to *Reticulitermes* spp. in the field. The pressure treatment resulted in an even distribution of boric acid throughout the wood. Based on early results, treatment solutions containing 0.5 percent TIM-BOR and possibly less, protected wood from these termites in laboratory tests. Chemical analyses and field results are not yet complete.

Variables which Affect the Acceptance of *Apis mellifera* Queens by Africanized Honeybee Workers

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Several studies on the introduction of queens in European colonies have shown that different factors may affect the degree of acceptance of new queens by workers. The lack of knowledge on the behavior of Africanized honeybees makes it difficult to conduct specific studies to improve their management and production.

The objective of this study was to determine the methodology more suitable to introduce Africanized (*A.mellifera adansonii*, hybrids) and Italian queens (*A.mellifera ligustica*) into colonies of Africanized bees.

MATERIAL AND METHODS

Two Langstroth hives for queen rearing, two observation hives and ten-three frames nuclei with Doolittle feeder each were used. 105 queens were introduced, of these 52 were Italians and 53 were Africanized. Different cages for queen introduction were used.

The experiment was done at the Laboratory of Bee Genetics-USP, in Ribeirão Preto, Brazil and was conducted during one year. The queens were previously selected by their strain, age, weight and state (either virgin or mated). The queens were marked on their thoraces with plastic ink and kept in different types of cages. The colonies received queens after being kept queenright or queenless for periods of 0, 24, 48 and 72 hours and according to colony size. During the introduction of the queens the observer remained in front of the cage for periods between 2-6 hours continually and for periods between 6-24 hours with intervals. This was accomplished for 73 of the 105 queens introduced. The behavior of queens and workers were noted. When the queens were accepted during the introduction they were allowed to remain in the hive for 4-5 days in order to check the acceptance. The data were analysed using Chi-square and G test.

RESULTS

Both Italian and Africanized queens may be introduced in Africanized honeybee colonies with similar probabilities of being either accepted or rejected. Mated queens had better probabilities of being accepted (88%-100%) than virgin queens (48%-63%). Queens between 0-5 days old had the best acceptance. Africanized queens were more attractive than European queens but Italian queens were more active during introduction. Queens that remained 48 hours in the introduction cages before their release - had the best acceptance (62% to 72%). The colony receiving the new queen should remain queenless at least 24 hours before introduction. The colony size did not have statistical significance on the acceptance. Queens should be introduced using an indirect way (use of cages) in order to allow a good contact between queen and workers (antennal contact etc.). (Research supported by CNPq-PIG).-

Do Fungicides Increase the Toxicity of Low Dosages of Insecticides to Honeybees (*Apis mellifera*) if Applied Together in a Mixture?

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To investigate the influence of fungicides on the acute toxicity of some commonly used insecticides, larval and adult worker honeybees were fed with fungicides and insecticides alone and with combinations. The insecticides were used at dosages that resulted in low bee mortality while the fungicides themselves were almost nontoxic to honeybees.

As a convenient measure of any change of toxicity of a pesticide combination for bees, the mortality (in %) observed after application of the insecticide-fungicide-mixture was divided by the mortality caused by the insecticide alone.

With both the *Apis*-larvae-test and the feeding-test of adult bees, it could be shown that the toxicity of all insecticides tested was significantly increased by the fungicides added (Tab.1, Tab.2).

Insecticide-fungicide-combinations	Number of combined concentrations	Total number of larvae N	n	$\frac{I + F}{I}$ \bar{x}	s
Rubitox - Pomuran	6	2372	33	4.60	2.7
Rubitox - Ronilan	4	1072	20	1.88	1.0
Orthen - Pomuran	4	1268	24	33.36	28.9
Orthen - Ronilan	4	1073	20	8.62	11.3

Tab.1: Mortality of insecticide-fungicide-combinations to honeybee larvae. The change of toxicity is expressed with the factor $I+F/I$.

Insecticide-fungicide-combinations	Number of combined concentrations	Total number of adult honey bees	n	$\frac{I + F}{I}$ \bar{x}	s
Rubitox - Pomuran	14	780	14	3.14	1.9
Rubitox - Sufran	16	1350	22	1.59	0.5
Rospin - Pomuran	6	330	6	8.13	4.2

Tab.2: Mortality of insecticide-fungicide-combinations to adult honeybees compared to the mortality of the insecticide alone. The change of toxicity is expressed with the factor $I+F/I$.

These data show that the simultaneous application of untoxic dosages of insecticides and fungicides results in a significantly enhanced toxicity of the mixture. Such effects may explain part of the bee-losses in fruit-and viticulture.

Film Documentation of the Traditional Heather Skep Beekeeping in Northern Germany

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In Northern, Western and Central Europe skep beekeeping is a tradition of at least 2000 years. Baskets made of wickerwork or straw and sometimes impregnated with clay or cowdung were the first bee hives used by the Germanic tribes. There was little change in beekeeping technology over the centuries until the movable frame was invented which in combination with strain equipment allowed to render honey much easier. Although this technique was made available more than 100 years ago, modern box and magazine hives did replace the old skeps only very slowly. Still in the fifties thousands of straw skeps were found in continental heather areas, especially to produce the very much appreciated heather comb honey.

For many reasons there was then a sudden change which led to the disappearance of most of the skeps within the last two decades. Mainly a number of old beekeepers still continue to work with skeps even in the Lüneburger Heide, a region famous for sheep-breeding and skep-beekeeping. Faced with the danger of a possible extinction of one of the oldest traditions and technologies of European beekeeping, we initiated a film documentation. This was realized during the past 10 years in the last remaining large-scale skep apiary located in the heather moor region in Northern Germany. This private enterprise is operated by the Klindworth family and is situated between Bremen and Hamburg. Depending on the season, about 1000 colonies hived in hand-made straw skeps plastered with cowdung are still in use there.

A series of 16 mm color films was produced in German and English versions. In each film a period of the routine work processes in a skep apiary is shown, altogether presenting the heather skep beekeeping in the course of the year. The aim of the film series is to document original beekeepers equipment and work from the viewpoint of ethno-entomology.

The films are published by the German Institute for the Scientific Film (IWF), Division of Ethnology, at Göttingen, tel. (0551) 21034, telex 96691. Copies are purchased and can also be rented free of charge for scientific teaching. The schedule of the IWF documentation No. 2025 Heather Skep Beekeeping in Central Europe, Northern Lower Saxony (Heideimkerei in Mitteleuropa, Nördliches Niedersachsen) with the IWF film list numbers and the titles of the 8 units completed until now are:

- E 2879 Spring Work in a Heather Skep Apiary
- E 2901 Preparations for the Swarming Period in a Heather Skep Apiary
- E 2946 Work in a Heather Skep Apiary during the Prime Swarming Period
- E 2962 Work in a Heather Skep Apiary during the Cast Swarming Period
- E 2994 Summer Work in a Heather Skep Apiary
- E 2790 Autumn Work in a Heather Skep Apiary
- E 2802 Harvest of Heather Honey in a Skep Apiary
- E 2661 Bees' Wax Pressing in a Heather Skep Apiary

As is usual for the Encyclopaedia Cinematographica there is an accompanying publication with every film, including technical data, an abstract and the complete tape recording and commentary on the film. A summary in brochure form on the whole film documentation is in preparation.

Exploitation and Protection Measures of *Apis florea* L. in Iran

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The occurrence of *A. florea* in Iran has been reported for the first time by Tirgari at the 23. International Apicultural Congress at Moscow (1971). Accounts were given on its local migration as well. Forced migration and torchering the bees is very common. This is due to the lack of knowledge on the biology and the lack of procedures for collecting the honey reserves. In a field experiment in late September 1985, a colony of *A. florea* was forced to leave its comb temporarily. The branch of the tree holding the bulk of the honey was cut away and the remaining part of the comb, including the brood rearing and queen cells, was replaced at the original spot. The colony returned to its original site and, within one hour, repaired its damaged comb. The colony continued brood rearing and other activities for several weeks. This observation may demonstrate:

- 1 - how to prevent destruction of the colonies and how to encourage the rate of their increase.
- 2 - the proper way of collecting honey, even two times a year.

The Establishment of the First Iranian Population of Gregarious *Megachile rotundata* (Fabricius), (Hym., Megachilidae)

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During the years of 1983-84, several thousands of special trap-nests were made. They were wooden blocks of different sorts and sizes, including lamellated blocks. The trap-nests were distributed in different parts of the country, in localities that are well known for alfalfa seed production. Thousands of leaf-cells containing full-grown *M. rotundata* larvae (prepupae) were collected from the traps. They were overwintered (6 months) and incubated (20 days) and then moved next to an alfalfa field (in July 1985) when it was in full bloom. At four mobile shelters, more than 20 000 gregarious adult females emerged and nested close together in 6 mm wide and 8 mm deep holes. The shelters were moved during the night to other spots at the end of the flowering period and the bees continued to nest without any obvious disturbances. In a similar way, a population of *Anthidium florentinum* (Hym. Megachilidae) was set up - for the first time, as far as we know.

Varroa jacobsoni (OUD) Changes its Host

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Varroa jacobsoni was detected first in Austria at the beginning of the 80s. In autumn 1985 the first large damages of bee colonies were reported. The mite lives ectoparasitic on bees and brood and is sucking haemolymph. We have studied if mites being on hatching bees are changing their host.

MATERIAL AND METHODS

Experiments were done in the south of Styria from May till August 1985. In infested and noninfested hives (with 10 to 30 frames) the queens were caged on an empty comb for one day. After 20 days these brood combs were put into an incubator and kept at $34 \pm 0.5^\circ\text{C}$ and $60 \pm 10\%$ relative humidity. The hatching bees were checked for mites and individually marked.

In the first experimental design three groups of 50 bees - infested bees, their noninfested sisters of the same comb and noninfested bees out of a noninfested hive - were put into one observation hive. This observation hive was not infested and contained about 3000 bees, one honeycomb and one comb with brood. This design was performed 7 times with unsealed brood (larvae from 1-9 day) and 3 times with exclusive sealed brood on the brood combs. Two hives were always observed in parallel.

In a second experimental design individually marked, just hatched infested bees were put into the infested large hive from which they had been removed.

At least 50% of the marked bees were investigated at the second day and those in the large hive also about 10 days later.

Sheets of plastic were placed on the bottom of the hives from time to time to see that no drastic loss of mites had influenced the results.

Significances were tested with the nonparametric U-test.

RESULTS

Originally infested bees in the observation hives with unsealed and sealed brood were infested on the second day at a rate of $12.0 \pm 2.6\%$, those in hives with only sealed brood at $17.7 \pm 3.7\%$, the originally noninfested control bees at $4.8 \pm 1.3\%$ and $3.3 \pm 1.9\%$, respectively (Table 1). The infestation of the originally infested bees was significantly higher than that of the noninfested ($p < 0.05$).

Results were similar in the second type of experiment. The average infestation was $14.2 \pm 2.4\%$ at the first and $5.4 \pm 0.6\%$ at the second control (Table 2). 88% of the bees found to be infested at the second control were infested at the first control. These bees infested again at an age of about two weeks are, with respect to their hormonal status, described to be the best hosts for the mites before the latter go into cells for reproduction (Hänel, 1984).

These results show a rapid host changing both in large hives and in observation hives. A dependence of the mite's leaving their first host on the existence of unsealed brood is small or nonexistent. This indicates a changing between adult bees. This behaviour of *Varroa* might be a source of spreading infections within the hive.

No. of Exp.	Date of control	unsealed brood existing	Group 1	Group 2	Group 3
1	9th May	+	12.4%	3.6%	0.0%
2	13th May	+	16.7%	11.5%	13.6%
3	14th June	+	4.0%	0.0%	3.6%
4	14th June	+	3.9%	0.0%	0.0%
5	5th July	-	13.8%	0.0%	0.0%
6	5th July	+	8.3%	0.0%	4.6%
7	1th Aug.	-	25.0%	12.0%	0.0%
8	1th Aug.	+	17.7%	6.9%	9.1%
9	10th Aug.	+	21.4%	9.7%	4.8%
10	10th Aug.	-	14.3%	3.6%	4.4%

Tab. 1. -- Percent of infestation of bees two days after hatching.

Group 1: Bees that were infested when put into the hive with 1 to 2 mites.

Group 2: Originally noninfested sisters of group 1

Group 3: Originally noninfested bees from another hive

No. of Exp.	Date of hatching	first control after	rate of infestation	second control after	rate of infestation
1	4th July	2d	12.6%	14d	6.4%
2	17th July	2d	8.3%	14d	4.2%
3	30th July	2d	16.5%	11d	5.7%
4	9th Aug.	2d	19.5%	---	---

Tab. 2. -- Percent of infestation of bees that had been put into an infested hive at different periods after hatching. Each bee put into the hive was infested with one mite. For one experiment at least 100 bees were used.

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Economic Evaluation of Beekeeping in Iran and the Estimate of Damage Caused by *Varroa* in 1985

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Beekeeping, by producing honey and contribution to crop pollination, is an important branch of agriculture. Reports show that in the last thirty years beekeeping has changed and developed both in developed and developing countries, especially in the tropics (Crane, 1980). The increase in world honey production from 600 000 tons in 1975 to 940 000 tons in 1984 (Bee World 1985) also confirms the above fact.

Iran is one of the main lands of *Apis mellifera*. Beekeeping has a long history in this country and it has many areas with favourable condition for this purpose. Ministry of agriculture statistics show that more than a million hives are kept by 40 000 beekeepers. The number of colonies kept by a beekeeper varies between a dozen to up to 1000 colonies. During the last decade beekeeping has developed in Iran and has been accepted as a part time or full time occupation by both rural and urban people. Total annual honey production could be estimated at about 8000 tons.

Presenting detailed and reliable information on costs and benefits of this industry would be a useful guide for newcomers, aid agencies, agricultural banks, etc. This investigation would also evaluate the economy of this industry in Iran and clarify how much resources could be allocated to *Varroa* control to have an economically justified industry.

MATERIALS AND METHODS

From the financial reports of eight beekeeping cooperatives, each with 3 - 4 members and 300 - 450 colonies, the annual expenditures, the annual income from honey, the increase, and finally the yearly income, based on four years period, were calculated. For the estimate of damage caused by *Varroa*, 235 apiaries with a total of 55 260 colonies were visited from Oct. to Jan 1985 and counts were made of the losses. On the basis of this figure, the total percentage of colony losses in Iran was estimated.

RESULTS AND DISCUSSION

The average of main current expenditures of each cooperative was as follows: transportation 200 000 Rials/year; Sugar & medicine 1000 Rials/hive; hive, wax, etc. 600 000 - 900 000 Rials per year; depreciation 600 000 - 800 000 Rials/year. The wholesale price for honey for the last year was 1000 Rials/kg and subsequent years 1 400 Rials/kg. The sale price of a medium size increase was 25 000 Rials. The average yearly incomes of each cooperative vary from 2 245 250 to 5 420 250 Rials with an average of 3 407 125 Rials. This variation is mostly due to management system and even the most successful enterprise

could increase its production and income by adopting a more efficient system. The average monthly income of each member varies between 62 368 to 112 921 Rials, with an average of 79 788 Rials. This figure, as compared with the income of other branches of rural agriculture and relative to the time spent, is high. It is also comparable to the salary of a medium ranking government employee. The net income of each hive per year varies between 10 590 to 16 083 Rials, with an average of 12 455 Rials. In other words, a beekeeper with 100-150 colonies, on the average would have 1 245 500 to 1 868 000 Rials / year, which is enough for a medium standard of living.

Damage by *Varroa* invasion was first reported in 1984 in the northwest of Iran. Despite all the measures taken by the beekeepers, it caused heavy damage in late summer of 1985. The extent of this damage varied from place to place and even from beekeeper to beekeeper in the same area. For example, my observation in Khorramabbad (the study area), showed that on the average more than 64% of the colonies were lost and the remaining ones were so weak that they could hardly survive the winter, whereas in the same district there were beekeepers who had not lost even one colony. The losses imposed to migratory beekeepers were as follows:

Azarbaijan district, 14 518 out of 39 446 colonies in 162 apiaries (35% loss); Luristan and central parts, 7 579 out of 15 642 colonies in 61 apiaries (48% loss); Khuzistan 63 out of 172 colonies in 12 apiaries (36.6% loss).

According to these data, the percentage of losses for migratory beekeepers is on the average 40.1%. Considering the total number of modern and traditional hives in Iran (1 030 030) it could be estimated that 413 056 colonies are lost due to *Varroa* invasion. The total loss in terms of capital may be estimated to be 10 326 400 000 Rials, equivalent to 103 264 000 dollars. I believe this could be an underestimated figure, because losses of colonies in stationary apiaries and losses of crop pollination was not considered yet.

CONCLUSION

Summing up the costs and benefits of small scale beekeeping it could be concluded that this industry is profitable. Since the cost of *Varroa* control in relation to its revenue is marginal, allocation of any effort to *Varroa* control is justified, especially when taking into account the important role of honey bee in pollination.

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Interspecific Competition between *Tropilaelaps clarae* and *Varroa jacobsoni* in Honeybee Colonies, *Apis mellifera*

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Varroa jacobsoni and Tropilaelaps clarae are new parasites of the honeybee, Apis mellifera, in Southeast Asia. A non-adaptable host-parasite relationship can lead to considerable bee damage. Although Varroa jacobsoni is always present in areas where Tropilaelaps clarae is found, they could not be found (Woyke 1984) or could only be found in small numbers (Burgett 1983) of colonies strongly infested with Tropilaelaps clarae.

Materials and Methods

The experiments were carried out in Thailand in 1984. In order to determine the degree of infestation of the bee colonies of Apis mellifera with Varroa jacobsoni and Tropilaelaps clarae both adult bees and brood samples were examined. The infestation of adult bees could be calculated by treatment with a medicament. The number and stage of development of offspring as well as the sex of adult parasites was determined.

Results and Discussion

The rate of reproduction be determined in 82 worker cells for Varroa jacobsoni. The actual reproduction calculated for Varroa jacobsoni from reproducing mites and the number of offspring was 1.86. The value becomes 1.27 when calculated for all mites in the brood. The reproduction rates calculated for Varroa jacobsoni in Thailand are in agreement with those found in Europe (Schulz, 1984). The simultaneous infestation of a colony with Tropilaelaps clarae and Varroa jacobsoni has no influence upon the ability of Varroa jacobsoni to reproduce when propagation occurs in different brood cells.

102 cells could be evaluated in determining the reproduction rate of Tropilaelaps clarae. The actual reproduction was 1.39 and 1.02 when calculated for the total brood. The difference between both parasites here is especially large. Since many Varroa jacobsoni repeat egg-laying, more than one offspring can be produced while the females of Tropilaelaps clarae predominantly lay one female egg.

A comparison of the reproduction rates shows that Tropilaelaps clarae females produce significantly less offspring than Varroa females. Only 28% of the cells examined showed coinfection with both parasites. 10 cells of these contained infertile mites. The number is too small to be able to determine a mutual influence on the reproduction.

The distribution of parasites on the worker and drone brood, as well as adult bees, was examined in 15 colonies. All colonies had been treated every 10 days with Naphtalin for 2 - 3 years. The colonies were grouped according to the time of their last treatment. In the first group 3 colonies had been treated up to the date of examination. *Varroa* mites were found exclusively in both worker cells and drone cells. In the second group 8 colonies were treated 3 months before examination. The degree of infestation of *Varroa jacobsoni* remained approximately on the same niveau despite the 3 month pause in treatment. This was also true for adult bees infested with *Tropilaelaps clarae*. Yet the latter parasite could also be found in brood; but the degree of invasion of the worker brood and drone brood remained relatively small. Another colony had not been treated for 2 years. The degree of infestation of the worker brood with *Tropilaelaps clarae* increased further while that of *Varroa jacobsoni* clearly decreased. The total infestation rose to 25%. Another colony had been imported, as package bees from the U.S., two years earlier and had since remained untreated. The degree of infestation of the worker brood with *Tropilaelaps clarae* had decreased, yet the drone brood present was extremely infested there by increasing the total degree of infestation. Only a few infested cells with *Varroa jacobsoni* were found.

The repetative treatment with a contact acaricide generally led to a weakening of the *Tropilaelaps clarae* population. Yet a decrease in *Tropilaelaps clarae* lead to an increase in the population of *Varroa jacobsoni*. The repression of *Tropilaelaps clarae* by *Varroa jacobsoni* is clearly an artifact resulting from treatment. The reverse procedure was, in contrast, the natural one. A faster increase in the population of *Tropilaelaps clarae* can be explained by the fact that they survive outside of broodcells 1 to 2 days only (Woyke, 1985). So they have to enter broodcells for reproduction in this period. The female of *Varroa jacobsoni* and *Tropilaelaps clarae* was more frequently infertile in cells simultaneously infested with both parasites than in those only infested with 1 parasite. The chance for *Varroa jacobsoni* to reproduce becomes smaller with an increase in the population of *Tropilaelaps clarae*. This could be a reason for the repression of this parasite.

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Treatment of Honey Bee (*Apis mellifera*) Packages Infested with the Mite *Varroa jacobsoni*

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INTRODUCTION

The mite *Varroa jacobsoni* is a serious pest of the honey bee *Apis mellifera*. The only continents that are free of the pest are Australia and North America. Since the mite is expected to reach North America in the near future, methods of controlling the pest are being sought. In the United States, bees are sold principally in the form of packages. This beekeeping practice will spread the mite rapidly once it enters the country.

MATERIALS AND METHODS

This study investigated acaricides for the control of *V. jacobsoni* in honey bee packages. The eleven acaricides tested were fluvalinate (Spur), amitraz (Mitaban, Varamit and non-commercial evaporation band), dicofol (Kelthane), oxythioquinox (Morestan), propargite (Omite), cyhexatin (Plictran), binapacryl (Acracid 40 EC), dienochlor (Pentac), chlorobenzilate (Akar), and ethyl- and dimethyl-dodecamine (IPL12 and IPL13). The products were used on disposable bee packages containing 500 bees and incubated at 30°C. Mites falling from the treated packages were counted after a 24-hour incubation period. Surviving mites remaining on the bees were then counted.

RESULTS

Of the eleven products, only amitraz and fluvalinate were effective acaricides. Further tests with these two products showed that amitraz spray was 98% effective at concentrations of 250, 25 and 5 ppm, and was not toxic to bees. Amitraz evaporation band caused mite mortality ranging from 68-100%. Amitraz smoke strip was 99% effective in all of the trials. However, it was very toxic to bees.

Fluvalinate (Spur) spray was 98% effective at concentrations of 120, 12 and 1.2 ppm. At a concentration of 120 ppm, it was toxic to mixed-age and one day-old bees.

Queens treated with fluvalinate and amitraz laid a normal number of eggs that eclosed with the same frequency as those from non-treated controls.

Use of Host Hemolymph Proteins, Seasonal Reproduction and a Hypothesis on Nutritional Imprinting in the Honey Bee Mite, *Varroa jacobsoni*, on *Apis mellifera* and *Apis cerana*

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The ectoparasitic hemophagous mite *Varroa jacobsoni* was originally only to be found on the eastern hive bee, *Apis cerana*, in Asia. Since the western honey bee, *Apis mellifera*, was brought there, the mite also moved onto this European hive bee and spread almost worldwide, becoming a serious pest to the secondary host. The reason for this stems from some peculiarities in the reproductive biology of the mite which have not been completely resolved. *Varroa* only reproduces in capped bee brood cells, preferably in drone brood. Mite nutrition and utilization of host proteins were studied in order to gain a better understanding of the parasite-host relations. In contrast to reports on *Apis cerana*, in colonies of *Apis mellifera* worker brood is also parasitized and damaged. In the case of heavy worker infestation - which often occurs without mite control - severe colony losses are inevitable. Apparently the adult female mites behave somehow different in *Apis mellifera* colonies whilst identifying and selecting the optimal host for reproduction. It has been conjectured that perhaps under temperate climatic conditions worker brood is parasitized in the secondary host because drone brood is not always available. Therefore, *Varroa* reproduction was monitored in the tropical plains of North India in colonies of the natural host which contained drone brood all through the year. The results of both studies are combined here to propound a hypothesis on a possible significance of hemolymph type for nutritional imprinting and resulting host preference in the *Varroa* mite.

MATERIALS AND METHODS

All analyses with *Varroa* mites on the western honey bee were done with *Apis mellifera carnica* colonies in Germany. Details of the methods used may be found in the previous publications. Seasonal fluctuation of *Varroa* reproduction on the eastern honey bee was studied in an experimental apiary containing about 200 hived *Apis cerana indica* colonies at the Government Beekeeping Training Unit in Allahabad, India. The 6 inspected colonies were medium sized and had 5 - 7 frames. Drone brood was permanently produced. Brood cells were opened fortnightly.

RESULTS

Varroa has to be regarded as being extremely stenophagous. Only honey bee hemolymph is sucked by both adult and immature mites. Surprisingly the host hemolymph proteins are resorbed by the mite without digestive degradation (Tewarson, 1981). The proteolytic activity of the female mite intestine was found to be rather low and furthermore inhibited by *Apis* blood factors (Tewarson and Jany, 1982). Under experimental conditions non-host proteins are likewise taken up (Tewarson and Engels, 1982). The bee proteins are incorporated into growing mite oocytes (Tewarson, 1982b), probably as a type of heterospecific vitellogenin. *Apis* proteins can still be detected in *Varroa* eggs, embryos and nearly hatched unfed protonymphs (Tewarson, 1982a). Therefore, host hemolymph is not only the basis for nutrition, but host proteins are also important for reproduction of this parasite. Whether this dependency is an exclusive one or not has not yet been proved by experiments. Female mites when entering the

still uncapped bee brood cell containing a fifth instar larva hide there in the provisions on the cell ground. Jelly proteins of this larval food were detected by immunotechniques in the Varroa gut, but it remains unsolved whether this material is used as a second type of nutrient or if just some of it penetrates into the intestinal tract of the mite. In Apis mellifera colonies the reproducing Varroa females invaded drone brood if available 3-5 times more frequently than worker brood. Using an in vitro assay in which the larva itself was the only present target for host identification, about 2 out of 3 tested mites approached and mounted old drone and not worker larvae (Rosenkranz et al., 1984). So there must be larva-derived stimulus involved in specific host identification by the mite. In the Apis cerana colonies studied, drone brood was available throughout the year. Varroa females invaded drone combs all the time, but for unknown reasons only produced progeny during a reproductive season from February to July (Tab. 1). The higher incidence at this time may be the result of an increased mite population.

	1985					1986						
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Adult Females	1.4	2.0	0.8	0.6	0.5	1.7	4.7	8.5	5.4	7.0	3.0	3.0
Eggs	0	0	0	0	0	0	2.0	2.2	1.3	3.0	0.5	0
Protonymphs	0	0	0	0	0	0	3.0	2.3	2.2	2.3	0.8	0.2
Deutonymphs	0	0	0	0	0	0	1.3	2.0	1.4	1.3	1.3	0.5
Adult Males	0	0	0	0	0	0	0.6	1.4	0.6	0.8	0.2	0

Table 1: Incidence (%) of adult and immature stages of Varroa jacobsoni in drone brood cells of Apis cerana about 4 days after capping. From 6 colonies 100 sealed drone cells were inspected twice per month. Therefore, the data represent average percentage of mite occurrence in 1200 bee brood cells. Notice the complete lacking of reproduction from August to January.

DISCUSSION

Reproducing Varroa females are trapped in Apis brood cells. At first the mites submerge into the larval food and are fixed there. Then after consumption of the last provisions by the bee larva, the cell is meanwhile sealed and opened again only by the emerging bee imago. During this period, hemolymph of the metamorphosing late larva and pupa is the only food available for the female mite and her offspring. Perhaps this nutritional dependence has some imprinting effect on the mites. Those growing up in a drone cell might at a later stage preferentially enter into drone brood again. Such a reproductive strategy (Tewarson, 1987) would enable the parasite to select the optimal host stage. As pupal drone development exceeds that of workers by several days, reproduction on male hosts gives the chance for more female progeny to reach adulthood. At the same time drone parasitism does not weaken the colony very much. Both circumstances might result in strong selective pressure. Within the social life cycle of the secondary host, Apis mellifera, long broodless periods occur during which the adult mites can only suck hemolymph from adult worker bees. Perhaps again imprinting effects later on guide some of the Varroa females to enter worker brood cells. If such a less discriminative behavior is indeed responsible for the damage caused by varroaosis, has of course to be confirmed by further studies. Possibly the reduced virulence observed in Africanized bees in Brazil could also establish because there drone brood is available to the mite throughout the year.

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Ecological and Behavioural Studies of Certain Olive Tree Borers and their Parasites in Egypt

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Stem borers such as the olive shot-hole borer, *Phloeotribus scarabaeoides* Berm. and the leopard borer, *Zeuzera pyrina* L. are the most serious pests attacking olive trees in Egypt. Therefore, this study was undertaken to contribute a better knowledge of the population fluctuations and the behaviour of these borers and their parasites.

A. Seasonal fluctuations: Adults of *Ph. scarabaeoides* begin to emerge in the 1st week of May till the 3rd week of December. Four borers could be detected throughout the following periods: April-July; June-November; September-November and November-December. Emergence of *Z. pyrina* moths started to appear in the 2nd half of April and continued till December; during this period, four broods could be observed. East-northern direction of olive trees was preferable for moth emergence. Temperature proved to be the principal prevailing weather factor that influenced borer activity. Relative humidity had a lesser effect.

B. Behavioural studies of *Ph. scarabaeoides*:

1. Mating behaviour: Soon after emergence, the female bores its main tunnel beneath the tree bark. It protrudes the end of its body out of the entrance hole, waiting for males which are aggregating outside. One of these males rushes into the entrance hole to copulate with the female.
2. Egg laying behaviour: After mating, the female works to excavate the egg gallery which is bimarous. The female lays its eggs in small cup niches on both sides of the egg gallery. Each niche includes one egg.
3. Larval behaviour: Immediately after hatching, the larva of *Ph. scarabaeoides* starts boring its gallery at a more or less right angle from the egg gallery. Each larva feeds singly in a separate tunnel, and remains in it until it becomes full-grown. When the larva reaches maturity, it makes an oval cell at the end of the larval gallery in which it will pupate.

C. Olive shot-hole borer/parasites relationship: Seven hymenopterous species of *Ph. scarabaeoides* parasites were first recorded in Egypt. All are ecto-prepupal and pupal parasites.

The present investigation clearly shows that high population densities of the parasites were always associated with high population densities of their host on olive trees. There was a highly significant correlation between the periods of activity of these parasites and their host.

3.2 Caste Differentiation

and

4.3 Social Behaviour

SYMPOSIUM:

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SYMPOSIUM*

Biology and Ecology of Neotropical Bees, Especially Stingless Bees

Organizers: Vera L. Imperatriz-Fonseca, Wolf Engels,
and Klaus Hartfelder

* The symposium consisted of two sessions:

- I. Development, distribution and taxonomy** (Section 3.2)
- II. Behaviour** (Section 4.3)

Introduction

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During the last few years a considerable number of papers was published presenting new results and insights into the biology of neotropical bees. In an effort to summarize all this accumulated knowledge in a comprehensive manner, we decided to organize a symposium especially dedicated to studies on neotropical bees with a particular emphasis on the highly eusocial Meliponinae. Unlike most of the sessions of this congress, we did not strictly adopt a topic-orientated grouping, but tried to integrate all topics related to the biology of the neotropical bees into one symposium. Nevertheless, as it turned out essentially two thematic fields ultimately received most attention. The first one comprises behavioral aspects of foraging, orientation and phenology, while the second concentrates on reproductive biology and caste development. It also became evident during the talk of J.S. Moure that the taxonomy of the stingless bees or the Meliponinae still presents a large number of undescribed species and unresolved questions. A new perspective on the evaluation of the chemical composition of larval provisions from a taxonomic point of view was given in a poster by K. Hartfelder.

The efficiency of two different foraging strategies - the "solitarily" and widely dispersed foraging pattern of *Trigona fulviventris*, and the "aggressive group" foraging strategy of *Trigona silvestriana* - was analysed by L. Johnson. For a number of stingless bees, solitary bees and the honey bee, interactions among the various species were studied from the ecological aspect by the group, L. Bego, V. Imperatriz-Fonseca, A. Kleinert-Giovannini and F. Knoll. In several contributions phenology, relative abundance, seasonality and floral resource sharing had been monitored on study sites in the state of Sao Paulo, Brazil. Aspects of the visual orientation of returning foragers of the stingless bee *Tetragonisca angustula* had been studied by J. Zeil and D. Wittmann, demonstrating the importance of landmark orientation in this species. The returning foragers use angles between landmarks as cues for finding the nest entrance.

In the monogynous colonies of the Meliponinae, intricate behavioral interactions can be observed amongst workers, of workers towards virgin queens, workers towards physogastric queens, and of virgin queens toward the physogastric queen. For a number of species, especially for the genus *Plebeia*, these various interactions which occur during maturation of virgin queens and subsequent return to the monogynous condition by either the killing of supernumerary gynes, supersedure of the old queen or swarming, were described by V. Imperatriz-Fonseca. Changes in odor-dependent gyne attractiveness are generally assumed to constitute the background for these behavioral events. Data presented by E. Engels, W. Engels and W. Francke on developmental changes in the cephalic pheromone bouquet of queens during maturation could corroborate this hypothesis. Evidently old physogastric queens produce a pattern of volatiles quite different from that of young virgins. Reproductive dominance of the egg laying queen in stingless

bees becomes most explicit during the complex behavioral interactions in the provisioning-oviposition process. Comparative studies of this highly species-specific process were presented in the films made by W. Hödl, W.E. Kerr and C.M. Schlichting, by J. van der Bloom, M.J. Sommeijer and by R. Darchen, in a paper of F. Benthem which was read by H. Velthuis, and in a poster by J. Mulder and M.J. Sommeijer. Morphological differences in the chorion structure of worker-laid reproductive eggs and trophic eggs were demonstrated by D. Koedam, M.R. Dohmen and M.J. Sommeijer. Results of another rarely studied phenomenon in colony homeostasis were given by P. Rosenkranz, W. Engels, E. Engels and S. Kläger in their poster on thermoregulation in stingless bee nests. In Scaptotrigona postica depilis temperature control mainly effects the warming up of the brood nest.

With respect to mating biology, a comparative study of eye structures in the honey bee and in stingless bees was given by W. Engels, W. Ribi and E. Engels. The drone compound eye in honey bees was found to be specialized in visual queen detection during flight. Optimization strategies for the prolongation of male territorial mating flights in a carpenter bee were presented by D. Wittmann and E. Scholz, and were discussed in the context of the bionomy of five different xylocopine species occurring in Rio Grande do Sul by the same authors.

Caste and sex morphometry in adult stingless bees, honey bees and bumble bees was treated from the comparative aspect by W.E. Kerr. Data on the trophogenic basis and the endocrine regulation of caste differentiation in one of the commonly studied stingless bee species presented by K. Hartfelder showed that the JH synthesis activity in queen corpora allata is distinctly higher during a critical period in the last larval instar.

Faunistic studies on bees for a long time have been treated like a step-child, and thus it was very rewarding to listen to the results of D. Wittmann and his students - B. Blochtein, M. Hoffmann, R. Radtke and E. Scholz - on the distribution and seasonality of euglossine bees and correlated chemical bait preferences in the males in South Brazil. This region is the southern geographical limit of this neotropical group.

In a paper of D.A. Posey, read by W. Engels, the fundamental importance of social insects for the cosmology and the living resources was reported for the Kayapo Indians of the Brazilian Amazone. Ethno-entomological research revealed detailed knowledge of tribe specialists on insect morphology, development and social life as well as indigenous insect use for nutrition, medicine and crop protection.

The study of neotropical bees has proved to be an open field for current research extending from the completion of the bee species inventory from the ecological aspect in areas widely threatened by man-made habitat changes or even by devastation, to behavioral and physiological investigations in the groups ranging from solitary to highly eusocial colonial life. Up to now the melittological knowledge accumulated in the indigenous indian cultures has scarcely been taken into consideration, but undoubtedly requires our scientific attention before it imminent irrevocable extinctions. Perhaps this symposium provided motivation to continue or initiate bee research projects in the yet largely unexplored neotropics.

The symposium consisted of two afternoon sessions chaired by H.H.W. Velthuis, D. Wittmann and V.L. Imperatriz-Fonseca, and also a poster exhibition.

Caste Differentiation in Stingless Bees: the Concept of "Nutritional Balance" from a Taxonomic Point of View

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Studies on problems concerning the trophogenic basis of caste differentiation have almost exclusively been carried out on a single species, *Apis mellifera*. As a consensus from a large number of feeding studies and analyses of larval food the following hypothesis has gained almost unanimous acceptance: "Queen differentiation is dependent on a nutritional balance in larval food." In our view the elusive logical structure of the concept "nutritional balance presents a problem, and is responsible for the low heuristic value of the above hypothesis. Apart from considering functional aspects of larval nutrition - i.e. sustaining larval growth, providing energy and metabolites for metamorphosis and, as in the case of caste polymorphism in bees, guaranteeing queen development - the species specific composition of larval food can also be examined from an evolutionary point of view. And, it is this latter aspect which provides the possibility to put a function independent test to the concept of nutritional balance.

Materials and Methods

The data from a comparative analysis of larval food from 7 stingless bee species (c.f. poster "Caste differentiation in stingless bees: A comparative analysis of larval food from 7 species", this volume) was used in this test. Three different categories were considered for each species: total composition of larval food (water, sugar, protein and free amino acid content), free amino acid composition, and polypeptide pattern. For each species pair the distance values in each of these 3 categories were calculated using two different distance algorithms. From the distance matrices minimal length trees were calculated by a Fitch-Margoliash least square method contained in the program package PHYLIP which had been written by J. Felsenstein. The minimal length trees for larval food were compared to the existent phylogenetic trees for stingless bees.

Results

For all three larval food categories the two distance algorithms resulted in congruent tree structures. Only the trees for total composition of larval food and the protein pattern, however, were consistent with the phylogenetic tree. For none of the 3 larval food categories excessive degrees of homoplasy could be detected.

Conclusions

Only with respect to the categories "total composition of larval food" and "protein pattern" our conditions for a "balanced composition" of larval food are fulfilled. The functional aspects of the larval food composition apparently do not impose evolutionary constraints on all components. Obviously the free amino acid composition - possibly due to the interconvertibility of some amino acids - is not governed by very strict selection.

Communication of Food Source Location by the Stingless Bee *Trigona fulviventrtris*

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The ability to communicate the location of good food sources allows *Trigona fulviventrtris fulviventrtris* Guérin (Apidae: Meliponinae) in Costa Rica (Tf) to exploit windfall resources before they become unavailable. I describe below features of communication and recruitment by Tf as a function of the sucrose molarity of experimental food dishes.

MATERIALS AND METHODS

The experiments were done in tropical dry forest, Guanacaste Prov., Costa Rica, May 24-June 23, 1978. Between 5:50 and 6:15 a.m. a food dish on a nylon campstool was erected 20 m in a varied direction from one of 4 Tf nests. The food dish was a sandwich box containing a 0.2-1.0 M solution of reagent sucrose and water scented with 240 μ l/l of methyl butyrate. A landing platform, incised with holes through which the bees could drink, floated on the solution. A leaf was placed in the corner of the dish for the scout to mark with pheromone from the mandibular gland. For each experiment a fresh landing platform was used; all other equipment was washed and heat-dried.

By 8:00 a.m., a scout of Tf would hover close to the set-up. To train the bee to the dish I held out a paintbrush dipped in sugar-water. The bee would land and be carried to the dish, where, if it drank (visit 1), I would mark it with paint. The behavior of the scout and all recruits was recorded for 2 hr after visit 1. In the 3 multiplicative recruitment experiments, the recruits were themselves allowed to recruit. At the end of 2 hr all visitors were collected. In the 9 non-multiplicative experiments, recruitment due directly to the scout was measured. All recruits were captured as they arrived.

RESULTS

In the non-multiplicative experiments, the lowest molarity (0.2M) did not elicit return visits by the scout. From 0.3-1.0 M revisiting occurred, and the number of recruits increased with molarity: 1, 0, 7, 10, 9, 6, 16, 17 at 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 M respectively ($r^2=.820$).

After the first taste, returning scouts made an orientation circle. Marking of the food source was stimulated by molarities from 0.5-1.0. In these cases the scout visited the dish 8-32 times simply to drink. On the first marking visit the scout would sample or fly over the food, then land restlessly for 1-2 sec on the food source or nearby prominent leaves, depositing bits of pheromone with the mandibles. Marks were concentrated around the food source; the only suggestion of a trail was a line of 8 marks 19.6-19.0 m from the nest, pointing at it. Of 277 recorded marks, 142 (51%) were at the food source, 119 (43%) were on leaves 0-100 cm from the food source, and 16 (6%) were on leaves

101-200 cm away. The number of marking visits made by those scouts that marked increased from 2-7 with molarity ($r^2=.778$), and the total marks recorded per scout ranged from 6-117, and tended to increase with molarity ($r^2=.498$). The number of the visit on which marking began ranged from 8-33 and showed no trend with respect to molarity.

Recruits were led in groups of 1-6 by the scout to the dish mainly in the first few visits subsequent to the first marking visit. The recruits hovered at the marks, which helped them locate the food. Occasionally a recruit accompanied the scout as it made a zig-zag flight in toward the food, before it had marked. A few other Tf flew in unaccompanied and hovered at the marks; it is not known if they were passers-by, or bees stimulated to search the vicinity of the nest for methyl butyrate. Twice the attracted bee was Trigona silvestriana, which landed on Tf marks laid 12 and 30 min earlier.

In the multiplicative experiments the number of recruits increased with molarity: 15, 122, and 152 bees at 0.3, 0.6 and 0.9 M respectively. The number of Tf at the dish increased stepwise as groups of up to 20 bees were brought in. The recruits sometimes marked before they themselves tasted the sugarwater.

DISCUSSION

Trigona fulviventris shares features of its communication and recruitment with other Trigona (Lindauer and Kerr, 1960), but at least with sources 20 m from the nest, does not lay a droplet trail between food source and nest, as do T. postica and T. bipunctata (Lindauer and Kerr, 1960, Kerr et al., 1963). Rather, the tendency of Trigona to concentrate marks around the food source is emphasized in Tf. Marking doesn't occur immediately as it would if its function were to "remind" the scout where the new food source is located. Instead, marking occurs just before recruitment and serves to direct the recruits to the food. Recruitment in Tf is delayed; the adaptive significance may be that after several visits the food source has proven it can provide at least several bee-loads.

The interest shown by T. silvestriana in Tf marks suggests the possibility that this species, an aggressive group forager that displaces Tf from resources, uses Tf pheromone as an indicator of potential food sources it can exploit.

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Stingless Bees and Africanized Honey Bees - Resource Sharing

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Some bionomic features, such as susceptibility to abiotic factors, size of individuals and colony population, influence foraging activity of social Apidae by restricting the period of flight activity (Imperatriz-Fonseca et al., 1985), home range (Free, 1970; Wille, 1983) and diversification of food gathering (Sommeijer et al., 1983). The efficiency of the communication system (Lindauer & Kerr, 1960; von Frisch, 1966), foraging strategies and colonial needs influence food utilization, which depends on floral diversity and presence of potential competitors. Here we evaluate the exploitation of floral resources by some social Apidae which reveal conspicuous differences in the above bionomic features. The study was carried out in a disturbed community with high floral diversity.

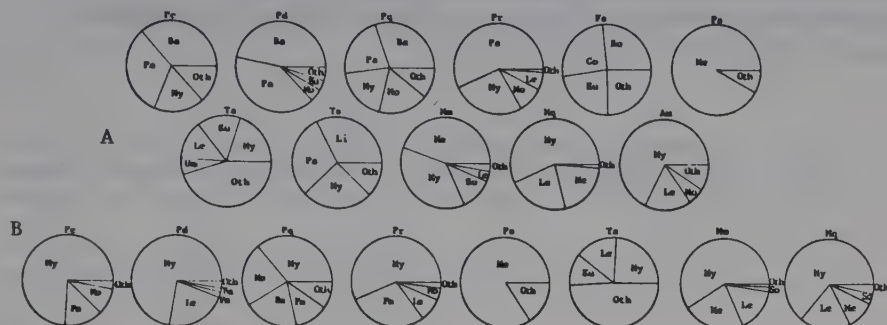
MATERIALS AND METHODS

Colonies were located in the gardens of the Biosciences Institute, University of Sao Paulo Campus (IBUSP). The floral composition of the area is varied, containing native and introduced species of plants and also a small semi-deciduous forest covering approximately 10 ha. Over a six month period pollen samples (3 to 4g) were taken from colonies of 10 stingless bee species *Plebeia* (P.) *droryana* Friese (Pd), *Plebeia* (P.) *emerina* Friese (Pe), *Plebeia* (P.) *remota* Holmberg (Pr), *Plebeia* (P.) *saiqui* Friese (Pq), *Plebeia* (Friesella) *schrottkyi* Friese (Fs), *Paratrigona subnuda* Moure (Ps), *Trigona* (*Tetragonisca*) *angustula* Latreille (Ta), *Trigona* (T.) *spinipes* Fabricius (Ts), *Melipona marginata* Lepeletier (Mm), *Melipona quadrifasciata* Lepeletier (Mq), and from one colony of Africanized honey bees *Apis mellifera* Linne (Am). From all these species - except Fs, Ts and Am - honey samples (4 ml) were also taken. The samples were acetolyzed and the frequency of pollen types was estimated, as described by Kleinert-Giovannini et al. (1986).

RESULTS AND DISCUSSION

Among the species analysed, the greatest pollen spectrum was observed for Ts, Am and Ta. These species have the largest colonies among the bees coexisting in the study area, thus confirming the finding of Sommeijer et al. (1983), who noted that for some species of stingless bees and *Apis mellifera* studied in Surinam, the diversity of the pollen spectrum was related to the colony population rather than to the body size of the individuals. From the 73 botanic families of which plant species occur in the IBUSP gardens (Fukushima-Hein, pers. com.), only few (12) were visited with high frequency by bees of the species studied by us (Fig. 1). Some of these were found to exhibit a high degree of similarity in plant taxa exploitation: Foraging bees of colonies belonging to the genus *Plebeia* mainly visited plant species belonging to the Balsaminaceae and Palmae. The latter family was also found in high frequency in Ts samples. Among this group, Fs should be mentioned for its differential exploitation of the resources. During the whole study period Fs only once visited (at a frequency higher than 10%) the same source as Ta, namely *Alchornea sidaefolia*

(Euphorbiaceae). *Melipona marginata* and *Melipona quadrifasciata* were also not worthy for foraging in flowers of Solanaceae and Melastomataceae species which require behavioral specialization for pollen collecting (Michener, 1962; Laroca, 1970). Ps is known to visit plants of the latter family harvesting the pollen left behind by larger bees (Laroca, 1970).



BALSAMINACEAE (Ba); BOMBACACEAE (Bo); COMPOSITAE (Co); EUPHORBIACEAE (Eu); LEGUMINOSAE (Le); LILIACEAE (Li); MELASTOMATACEAE (Me); MORACEAE (Mo); MYRTACEAE (My); PALMAE (Pa); SOLANACEAE (So); UMBELLIFERAE (Um); OTHERS (OTH).

Fig. 1 -- Pie diagram of the most represented families in pollen (A) and honey (B) samples.

For all bee species (except Ps) Myrtaceae was the family most represented in honey samples, mainly due to the high frequency of *Eucalyptus* during the last four months of the study period. This genus was also intensively exploited by Am and Mq for pollen harvest. In all months few sources were intensively exploited by each bee species, and a certain degree of similarity in floral use between various colonies of the same species was observed. Generally speaking the degree of intraspecific similarity was higher than the interspecific one. Our data suggests that there is a floral preference by the eusocial bees in this community. Some sources - such as *Eucalyptus* spp. which is very productive - were either exploited by Am and stingless bees, while others were of changing value for them. The differential use of resources might be related to foraging strategies and colony needs of the bee species studied by us.

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Relative Abundance and Phenology of Bees (*Hymenoptera*, *Apoidea*) in Sao Paulo, Brazil

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A number of factors influences bee foraging activities: temperature, relative humidity, light intensity, wind, rain, floral availability; a whole set of abiotic factors also influences the pollen and nectar production by plant species, being their attractiveness to the bees variable.

The survey of the bee species that share resources found in a same site provides the basis to the comprehension of the complex interrelationship that this coexistence brings on. Besides this, nowadays the interest in studies about any factor that plays a role in the survival and reproduction of insects in urban environments is increasing. Thus, the aim of this study was to provide data about the relative abundance and phenology of bee families in a modified environment in São Paulo city, Brazil.

MATERIAL AND METHODS

Study area: This work was done in an urban area at São Paulo University Campus, Brazil (23°33'S; 46°43'W). The climate is subtropical with rains homogeneously distributed along the year, with fresh winter and hot summer. Mean annual temperature and rainfall are 17,6°C and 142,8 mm, respectively. The hottest period is rainy (October to March) and in the beginning of winter there is a rainfall peak.

Sampling: During one year (July/81 to June/82), bees were regularly captured by means of random sweeping each 10 days, generally from 8: AM to 17: PM, in all herbs, shrubs and small trees. The methodology followed in this study was that of Sakagami et al. (1967).

RESULTS AND DISCUSSION

During the study year 8732 individuals, belonging to 133 bee species were captured. The most abundant family in number of species was Halictidae (49 sp), followed by Anthophoridae (36 sp), Megachilidae (20 sp), Apidae (14 sp), Colletidae (9 sp), Andrenidae (4 sp) and Oxaeidae (1 sp). In number of individuals the eusocial Apidae was the most abundant, with 87% of the total number of bees captured. The most frequent species were *Trigona spinipes*, *Apis mellifera*, *Tetragonisca angustula*, *Paratrigona subnuda* and *Nannotrigona testaceicornis*. (Table I).

Concerning the bee families' phenology, males and females of Apidae and Halictidae were collected on flowers all round the year. In general, males were more numerous than females in the latterst family, mainly from March to June. Anthophoridae males and females were captured in a similar ratio over all months, excepting October. Megachilidae females were collected during the whole year and males only from October to March and in May. This family was far more abundant in number of individuals from December until February.

The other bee families - Andrenidae, Collectidae and Oxaeidae - were less represented in samples, being captured mostly from December to June (Table I).

Generally speaking the period of greatest abundance was observed from November to June, with highest peaks in February and June.

The relative abundance of bee families in number of species in our study was very similar to that obtained by Sakagami et al. (1967) in São José dos Pinhais. The relative abundance of bee families in number of individuals was more similar to that verified by Heithaus (1979) in Guanacaste. This is probably related to the several kinds of human

interference in the habitats studied in Paraná, which alter the environments in several ways, modifying the relative success of resident groups.

TABLE I - Number of individuals and species of the bees sampled in São Paulo, 1981 - 1982. (* undetermined).

	ANDREN		ANTHOPH		APIDAE		COLLET		HALICT			MEGACH		OXAEIDAE	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	*	♂	♀	♂	♀
MONIHS															
VII			10	11	0	427	1		11	8	1	1	2	1	
VIII			7	8		418	1		4	15	2		2		
IX			1	7	2	321		3	7	16	1		1		
X					2	857		1	1	23	3	1	3		
XI		2	11	13	5	1207			13	44	3	1	2		
XII			10	6	2	786	2		24	21	0	1	27		
I	1	1	12	9	4	591			26	35	3	2	19		1
II			13	32	7	605	2	4	32	28	2	7	8	7	
III	2		5	9	5	365	2		74	24		1	7	4	1
IV			16	8	2	346	1		72	21	1		2		3
V			1	5	2	306	1		27	4		1	2		
VI		2	16	12	1	1333	5	2	188	36			4	2	1
Total Nº of ♂ and ♀	3	5	102	120	32	7562	15	10	479	275	16	14	79	14	6
Nº Ind.	8		222		7594		25		770			93		20	
Nº sp.	4		36		14		9		49			20		1	

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Abundance and Resources Visited by Individuals of *Tetragonisca angustula*

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Tetragonisca angustula is one of the most common species of stingless bees and one of the most largely distributed in Brazil. It is found in almost habitats, including those in urban centres. It is a generalist species in relation to the choice of nest sites and the use of food resources.

The aim of this study is to provide data about the relative abundance and resource utilization by this species in an area around the Bioscience Institute in São Paulo, city Brazil.

MATERIAL AND METHODS

During one year (July/81 to June/82), individuals of *T. angustula* were sampled in the flowers of the gardens around the Biosciences Institute (University of São Paulo, Brazil). Samples were taken each 10 days, mainly in herbs, shrubs and small trees. Bees were captured during 3 minutes in each isolated or aggregated plant species. Later the material they were carrying was identified; the bees that not presented pollen or resin were considered nectar gatherers.

RESULTS AND DISCUSSION

During a year 1206 individuals of *T. angustula* were captured. This species was the third more abundant in the study area, among 133 species of Apoidea, coming after *Trigona spinipes* and *Apis mellifera* (Knoll, 1985). A similar result was obtained by Cortopassi-Laurino (1982) who observed the bees on flowers in the same site but in a different period.

The abundance of *T. angustula* has varied according to daily meteorological conditions, mainly temperature and rainfall, and to the successive flowering of the plant species. Generally speaking, this species was more abundant in the hottest and most humid period, from October to February and in June.

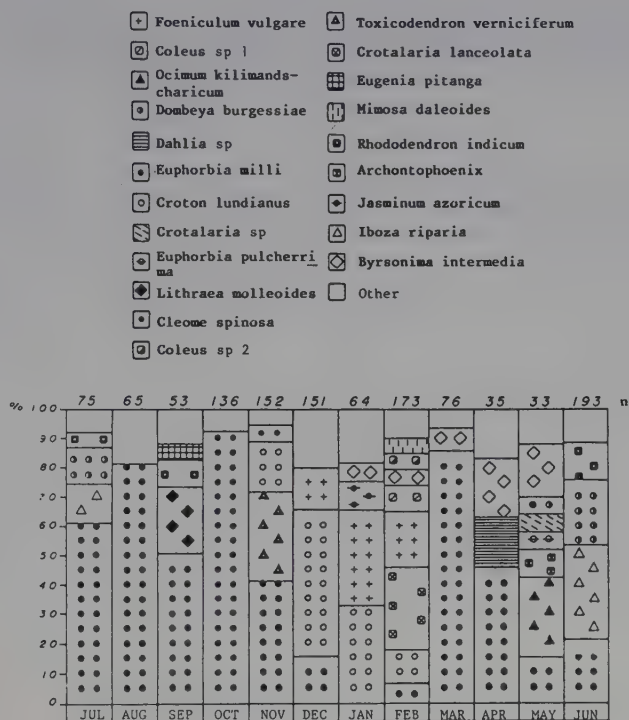
In spite of the great disponibility of plant species, *T. angustula* was captured in 69 species belonging to 32 botanic families, approximately 1/3 of the total sampled.

The most visited plant species were *Euphorbia milii* and *Croton lundianus*, both Euphorbiaceae. Labiatae, Leguminosae, Anacardiaceae and Umbelliferae were also well visited by the workers of *T. angustula*. The more important bee plants to are presented in Figure 1. From all captured *T. angustula*, 1127 individuals (93,4%) were examined in order to identify the material they were carrying. Most bees (50%) had empty corbicula and thus were considered nectar gatherers; 38,9% presented pollen and 11,1% resin.

Another possibility to evaluate the floral preferences of eusocial bees is the pollinic analysis of the food harvested by the bees. In *T. angustula* Iwama & Melhem (1979) analysed honey samples of colonies kept in the same region, and Imperatriz-Fonseca et al. (1984) analysed pollen and honey. In all these analysis, family Euphorbiaceae was the more important for these bees.

Our results are related to the local resources harvested by the whole population in the study area whereas pollen analysis focuses the food gathered by one or more studied colonies, in all home-range.

FIGURE 1 - Frequency of *Tetragonisca angustula* captured in the plants species (n = number of captured individuals).



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Visual Orientation towards the Nest in a Stingless Bee (*Tetragonisca angustula*)

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Tetragonisca angustula bees show two distinct and visually controlled orientation behaviours in the vicinity of their nest: as guard bees, they hover in very stable positions on both sides of and close to the nest entrance for up to 70 min, whereby for most of the time they face the flight corridor or the nest entrance. Hovering guard bees attack intruders and thereby discriminate visually and by olfaction between foraging nest mates and intruders (Wittmann, 1985). As foraging bees, they accurately locate the nest and the nest entrance when returning from foraging flights. We describe experiments designed to identify the cues used by the bees in nest orientation.

MATERIALS AND METHODS

Bees were kept in a wooden nest box (27cmx50cmx15cm) in the centre of a roof terrace (5mx5m) in Porto Alegre, Brazil. To study the visual orientation of guard bees towards the nest we filmed their flightpaths (16mm, 50 frames/s) while oscillating the whole nest box through 20 cm foreward and sideways with respect to the direction into which the nest entrance pointed. We reconstructed their flightpaths by single frame analysis of the films. We studied nest orientation of foragers by counting returning bees while displacing the nest and modifying landmarks in its vicinity. Our procedure was to count foragers entering the nest within 15s intervals for a total period of 12min which we subdivided into 1. a control period C1 (3min); 2. a test period T (3min) where we displaced the nest box for various distances (0.65m, 0.95m, 1.3m, 1.6m) foreward, backward or sideways with respect to the direction into which the nest entrance pointed; 3. a post test period PT (3min) immediately after returning the nest box to its original position; and 4. one further control period C2 (3-6min). To test whether bees use landmarks in order to locate their nest we carried out one experiment (C1,T,PT,C2) where during T the nest box was displaced 1.6m foreward followed by a second experiment (C1,T,PT,C2) where the nest box was again displaced 1.6m foreward but in addition during T and PT dominant landmarks were modified (the height of a wall behind the nest was increased and the vertical corner of a wall sideways from the nest entrance was moved foreward with large screens) so that the modified landmarks appeared at the same angular position at the displaced nest as the unmodified landmarks at the normal nest position.

RESULTS

When the nest box is oscillated at velocities up to 40cm/s hovering guard bees keep their position and distance relative to the nest entrance very accurately (to within 5-10cm) by flying foreward, backward and sideways while keeping the angular orientation of their body long axis constant for most of the time. In this ability they resemble hover-

flies (Collett and Land, 1975) much more than any other species of bee. Since they can voluntarily vary their orientation relative to the nest entrance, guard bees presumably use the retinal size and shape of the nest image to adjust their position. Guard bees sometimes follow incoming foraging bees by angular and sideways tracking along the last 15cm in front of the nest entrance. Keeping a constant distance and orientation relative to the nest entrance and to the flight corridor (and with this to the returning foragers) might help guard bees to discriminate between the different sizes of foragers and other insect intruders during tracking.

On the nest orientation of foraging bees we find that (1) when the nest box is displaced sideways, forward or backward through 0.95m or more, fewer bees return to the nest during the test period T compared to C1 and C2 (because the returning foragers search at the normal nest position) and much more during the first 30s in PT (because these searching bees now find the nest at its expected position). When the nest is displaced by 0.65m in either direction there is no such difference in numbers between C1,T,PT and C2. Our interpretation is that the nest box itself does not serve as a landmark for the bees until they are within about 0.6 to 0.9m from the nest. To within that range bees must be guided by other landmarks in the area around the nest. (2) To test this we modified two of the most prominent landmarks (both about 2.5m from the nest) and found that the bees find the displaced nest more easily when these landmarks are 'displaced' accordingly than when there is a mismatch between 'expected' landmark positions and the ones actually seen at the nest. (3) At less than 1m from the normal nest position bees accept any dark dummy object as the nest. They search for the nest entrance at the appropriate position and height. Bees have extreme difficulties of finding the nest entrance when the nest box is displaced 0.95m upwards.

The procedure we describe is simple and effective in studying nest orientation in stingless bees. Our results show that bees use landmarks in order to locate the position of their nest. Qualitative observations suggest that returning bees might use a sequence of 'snapshots' (Cartwright and Collett, 1983, Wehner, 1983) to find the nest (landmarks around the nest - nest box - nest entrance).

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The Role of Queens in Stingless Bee Colonies

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Studies about the role of queen in colonies of eusocial bees have focused their attention mainly on the queen's dominance and her control upon the ovarian development of workers and the appearance of new queens in the colony. In the more primitive social species the queen's dominance is achieved by behavioural interactions, while in the eusocial species it is attained by pheromonal control, although the two types are neither mutually exclusive nor sharply separated (Sakagami, 1982).

In stingless bees a very complex situation arises. Brood cells are constructed and used only once and mass provisioning and oviposition processes, carefully studied by Sakagami & Zucchi and cols (Sakagami, 1982), indicate a high evolutionary degree of sociality. An unique feature among social bees is the ovarian development of workers even in the presence of the queen in most species studied until now (e.g. Sakagami et al., 1963). The meaning of these workers' eggs is a central point on stingless bee sociobiology. Because of the mass provisioning it is also necessary to maintain a surplus of virgin queens in the colony to assure its perennity if the queen dies. Being the continuous production of gynes almost a rule in Meliponinae colonies, some similar mechanisms should be developed by the species to keep them alive in the presence of the queen. In this analysis of dominance subordination systems concerning the sociality of stingless bees and the role of queen, we will consider the interaction between virgin queen(s), queens and workers in the colony.

Just after hatching gynes can be or not attractive to the workers according to their taxa, but all of them need a maturation period before fertilization. Gynes which hatch attractive evoke a violent excitement on the workers, by the deliverance of pheromones that are rapidly spread through the colony. They are generally pigmented and run through the colony, vibrate wings, look for trophallaxis with workers, inflate abdomen and all this excitement disturb the oviposition process inside the colony. The balance between environmental condition and internal ones, generally the phase of colony's cycle and the physiological state of the queen, will determine the destiny of these gynes: to be killed or imprisoned by the workers.

The construction of a cerumen prison, generally at the pot region (Moure et al., 1958), can be observed in *Plebeia remota*, *Plebeia droryana*, *Plebeia emerina*, *Friesella schrottkyi*, *Friesemelitta varia* and *Friesemelitta silvestrii*. Observing the gyne behaviour inside these prisons, with the help of a glass slide, it can be seen that she opens or closes a hole in the prison to allow or not the workers to come inside. In this territory she delivers pheromones, has trophallaxis with workers or rests alone. A gradual increase of attractiveness is observed until the gyne abandons the prison and goes toward the comb region. This act is again accompanied by an increasing excitement of workers and can be considered the end of the maturation period.

Another kind of virgin queen imprisonment was observed by Terada (1974) in *Leurotrigona muelleri* and *Celetrigona longicornis*: workers encircle the virgin queen with a strong court and do not allow aggressive workers or the queen to arrive near the gyne until she completes her maturation phase.

In many species the gyne do not hatch attractive, joining the kindergarten assembly of workers: *Tetragonisca angustula*, *Tetragona clavipes*, *Scaptotrigona postica*, *Schwarziana quadripunctata*, *Partamona testacea*, *Paratrigona subnuda* and species of the genus *Melipona*. A sign of the beginning of attractiveness is the gyne being chased from the comb by some workers. Two types of behaviour can be observed:

a) as soon as attractiveness appears, the gyne is imprisoned in a cerumen prison: *Schwarziana quadripunctata*, *Tetragonisca angustula*.

In the examples above workers play a specific role by keeping the virgin queens away from the queen and comb. At this point it could be considered that there is a conflict between queen dominance, protection and gyne attractiveness; through workers informations about internal and external conditions are transmitted, determining the destiny of the gyne. b) gynes look by themselves for a refuge area where they will rest during the attractive period of their maturation phase. Two mechanisms were described:

1) The gynes, after becoming attractive, look for empty pots where to hide for some time. In these pots the gynes will rest and probably restore glandular secretions, which will be used in the contacts with workers. The time spent by the gynes and workers can vary. The gyne's pheromones are generally delivered outside the pot which can be occupied by successive gynes at the same day, suggesting the inexistence of individual scents in these pots. These gynes will complete their maturation phase when they arrive at the comb where they try to supersede the queen or go away with a swarm (Imperatriz-Fonseca, 1977),

2) Gynes of *Melipona marginata*, according to internal conditions of the colony, remain inside the nest looking for places where to hide, generally empty pots. Some of them look for a refuge, most of all on the boundaries of the nest. In these refuges, constructed probably by the workers, until three gynes were found together, exhibiting a dominance relationship between them, concerning food regurgitation observed during the trophallaxis processes, being the dominant always attended by the others which crouched in a typical subordination attitude.

Although differences are found on the routes followed by different stingless bee species to maintain virgin queens alive, some behavioural characteristics are common to all of them, mainly concerning the gradual increase of physical and pheromonal contacts between gynes and workers that translate the meaning of the maturation phase as a gradual acceptance process inside the nest. The main problems to be solved by the gynes are first related to the dominance of the physogastric queen of the colony, which stays generally on the brood comb, her main territory. In this maturation period gynes will keep contact with workers, develop glands and try to be safe to attain the goal of their lives: to be a queen.

Dominance in stingless bees is so linked with oviposition behaviour that it can be expected that a dominance competition should occur at the comb region, and that it could involve worker oviposition behaviour. So, a sign of acceptance and dominance is the possibility to eat workers' eggs, or trophic eggs, at the comb, without being molested by any worker. This fact should mean the conquest of the territory and the dominant position in the colony. These assumptions, based on several dispersal observations on stingless bees, are plausible if it is considered that attractive queens are not disturbed in relation to the oviposition process by the gynes which are still in their maturation phase.

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Do Stingless Bees have two Complexes of Queen Substances?

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The honey bee queen substance is known to be produced during the entire lifetime and to have polytropic functions. For the major compound of the mandibular gland secretion, 9-oxo-E-2-decenoic acid, six main functions can be specified: 1. Drone attraction during the mating flight. 2. Worker attraction within the hive. 3. Worker attraction during swarming. 4. Worker domination. 5. Prevention of royal cell building. 6. Inhibition of worker fertility. - Of these only male attraction represents an original female pheromone effect. All other functions may be considered as derived and the result of a socioevolution in which a permanent queen dominance was established. Since the stingless bees have achieved a similar level of social complexity, the question arises whether their social regulations are also effected by a queen substance.

MATERIALS AND METHODS

Stingless bees of the Brazilian species, *Scaptotrigona postica depilis*, were studied in the meliponary of the Dept. of Genetics, USP Campus of Ribeirao Preto, S.P. Young queens of known age were kept in nucs similar to filial nests. Gyne attractivity for the males was tested in a mating box bioassay (Engels and Engels, 1986), using groups of mature drones removed from an aggregation (Engels and Engels, 1984). Cephalic volatiles of individual queens were analysed in pentane extracts (Francke et al., 1983).

RESULTS

In *Scaptotrigona postica depilis* drone aggregations consisting of hundreds or thousands of males can be observed in the vicinity of colonies containing virgin queens. There is much evidence that such drone assemblies are stabilized by a male aggregation pheromone. 10-15 days after emergence young queens are most attractive for the drones. During this mating age the virgin is not very attractive for the workers within the colony, in contrast to the newly mated queen who is highly attractive. During a subsequent period of acceptance which lasts several days, she moves around within the brood area of the nest and by continuous wing fanning presumably disperses volatiles. About a week after the nuptial flight the queen starts ovipositioning and becomes physogastric.

Within the spectrum of cephalic volatiles in young virgin queens a number of secondary alcohols is predominant. Their peaks were recorded shortly before or during the mating age. A few days after mating, the concentrations of these compounds were found to be drastically reduced which is also true for old queens. In contrast, several alkenes which in virgin queens were either absent or detected only in trace amounts remarkably increase immediately after the mating age and become predominant in physogastric queens. These patterns of queen head volatiles greatly differ from age- and function-dependent changes described for workers in the same species (Francke et al., 1983).

DISCUSSION

Concerning possible queen substances, stingless bees apparently differ from honey bees in several aspects. Firstly, there is no compound chemically closely related to the major component of the mandibular gland secretion typical for all the recent *Apis* species (Shearer et al., 1970). Secondly, no single compound is found as a main pheromone constituent throughout the queen's life. Thirdly, there are pronounced qualitative and quantitative differences in the predominant volatiles found in young and old queens.

Therefore, it seems likely that stingless bees - at least the species studied by us - have two chemically and functionally different complexes of cephalic queen substances: One related to young queens consisting of secondary alcohols, and a second one effective during the physogastric status and made up by alkenes. This assumption of course still has to be verified experimentally.

Which of the polytropic functions of the honey bee queen substance occur in stingless bees? 1. Drone attraction is perhaps mediated by the secondary alcohols present in the virgin queen during the mating age. 2. Worker attraction within the colony could be attributed to the alkenes, as only a mated queen is attractive. 3. Worker attraction during swarming probably does not occur in stingless bees at all; the exodus to a filial nest is initiated by the workers which perhaps themselves by means of pheromones stimulate virgin queens to join the swarm. 4. Worker domination by the queen is less pronounced in stingless bees, but could be effected by the alkenes released by the old physogastric queen. 5. Royal cells are permanently built in queenright colonies, there is no evidence for a preventative factor. 6. All the workers are temporarily fertile in queenright colonies, apparently there is no inhibition effect.

The conclusion is that not all the social regulations, known to be queen-substance-controlled in honey bees, exist in stingless bees. Those observed are assumed to be released by the queen lure typical for old queens. The female sex pheromone apparently is not involved in domineering functions. The highly eusocial honey bees and stingless bees probably followed separate chemical courses in establishing a queen-dependent colonial homeostasis.

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FILM PRESENTATION:

**Oophagy and Oviposition in the Brazilian Stingless Bee
*Melipona compressipes fasciculata***

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Worker oviposition behaviour in stingless bees is characterized by a remarkable diversity, unknown in any other group of social insects. Worker eggs laid in queenright colonies are found in the majority of the stingless bee genera (Sakagami, 1982). In *Melipona*, trophic and male producing eggs are laid into the brood cell after food provisioning (Sakagami and Oniki, 1963; Sakagami et al., 1965).

MATERIALS AND METHODS

The oviposition process in the "tiuba" bee, *Melipona compressipes fasciculata*, was studied in observation hives at the Department of Biology in Sao Luis (Brazil). The behavioural activities on the brood comb were filmed with a 16 mm camera (ARRIFLEX 16 SR) during night hours, a period when all bees remain within the hive (Hödl et al., 1986).

RESULTS

The sequence of the food provisioning and oviposition process in *Melipona compressipes fasciculata* is stereotypic and repeated separately for each single cell. The horizontally arranged brood combs (between three and ten per hive) grow by successive additions of cells on the edges of the comb. Six to twelve workers participate in the formation of a new cell, whose construction lasts up to nine hours (cf. Sommeijer et al., 1982). Brood cells measure 12.2 mm in length and 5.5 mm in external diameter. Cell building continues calmly until the walls are completed. Then the workers crowd in and exhibit increased activity, and the queen is attracted. Workers finally form a frozen rosette around both the cell orifice and the queen, which remains quietly at the cell margin. During this fixation phase, between 6 and 15 workers provide the cell with 150 to 270 mg of larval food. After food provisioning is completed, usually one worker places an egg vertically on the center of the surface of the larval food. As soon as this worker leaves the cell the queen inserts her front body and consumes that egg. Trophic eggs are not ingested by workers. Oophagy is followed by queen oviposition: The queen mounts the cell and inserts her abdomen. Under heavy abdominal contractions and constant wing beating, the egg is laid within about twenty seconds. The size of the queen egg and its position in the brood cell does not differ from the one laid by a worker. After oviposition, the queen immediately leaves the site. The worker positioned behind the queen during the fixation

and food provisioning phase seals the cell. During the last phase of the four to five minute cell closing process she is usually substituted by another worker.

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FILM PRESENTATION:

Strategies of Reproductive Dominance in Stingless Bees, with Special Reference to *Melipona favosa*

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The film shows various aspects of intranidal behaviour of stingless bees the main emphasis being on queen-worker interactions. At the start of the typical provisioning and oviposition process the workers are stimulated by the queen to regurgitate liquid larval food into the brood cell.

Laying workers oviposit when the cell is filled with larval food. The queen ingests the worker egg prior to ovipositing. She also feeds on larval food that is discharged into the cell. In addition, she is fed directly via trophallaxis by the workers. Such trophallaxis occurs (as is also shown for *Hypotrigona braunsii*) just before the start of cell provisioning.

Laying workers in queenless colonies compete for ovipositions. The film also shows the aggressive behaviour of laying workers in a colony with a non-dominant virgin queen. Furthermore the morphological differences are shown between the eggs of workers that lay under such ("queenless") conditions and those of workers laying under normal queenright conditions. The various mechanisms for the execution of reproductive dominance are discussed.

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FILM PRESENTATION:

**Laying Ritual of a Queen, *Melipona beecheii*,
a Stingless Bee****Roger Darchen**

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INTRODUCTION. Some colleagues have described the laying behaviors of queens of the genus *Melipona* as well as comb building ones which are closely linked to them (see references). My own observations confirm some author's remarks (1983) on the uniformity of these stereotyped activities of both queen and workers of different species of the same genus of *Melipona*. Sometimes, some small behavioral details characteristically distinguish species.

In return, these till now described rituals in the Trigonini (see references) are so much diversified that they could help the taxonomist at least at the level of the genus.

All these considerations encouraged us to complete our filmotheca (see "*Apotrigona nebulata*, an african social bee") by "The Laying Ritual of a Queen, *Melipona beecheii*, a Stingless Bee"), reserving the publication of a more detailed analysis for the future.

MATERIAL AND METHODS. A small population (200 workers) of *M. b.* with some combs was deposited into an observation box (20x20x10 cm). Some animals were marked with colored tags with number codes. The behavior was recorded on color films and all the observations were written on cards.

OBSERVATIONS. During the extra-oviposition periods, the queen of *M. b.* rests on old combs surrounded by a small court. During this time, some workers build one type of cells for the males and the females.

The queen leaves her resting area and briefly hinges on new empty cells (brief fixations). She progressively runs on the combs trying to communicate her excitation to the whole population. The rate of trophallaxis slowly grows both in queen and workers. Her wing beats are accelerated. Then the queen definitively hinges on an empty cell. A small number of workers court her and begin to regurgitate food into the cell, while they are tapped by the antennae and the fore-legs of the queen. Sometimes the queen inserts her head into the cell to inspect the level of the food content. Suddenly, after a new visit she mounts the cell, inserts her abdomen into the hole left at the top of the cell and lays. It is not rare to see some workers laying eggs before the queen who eats them immediately. One single worker closes the completed cell. The queen leaves the comb, cleans herself, visits the closed cell and rests for the next behavioral cycles.

CONCLUSION. For one who knows the laying behavior of the queen of *Apis mellifica* this laying ritual shows a big complexity. The queen here seems to be more the chief of an orchestra whose signals (mechanical and chemical ones) always precede the behavior of the workers, except during the cell building phase. The behavior of both queen and workers is so perfectly synchronized that it drives the actors to the extinction of their behavioral impulses by the closure of the cell provided with

food and an egg. These behaviors are so well scored that we do not think that the behavior of laying workers is a kind of accident in the development of their play in the sense of a "rivalry" between them and their queen. On the contrary, these workers' layings appears as a behavior which is in the general unrolling of the laying ritual. This behavior strongly recalls the one of the laying ants which feed their queens and their larvae with special eggs.

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The Oviposition Process in *Plebeia remota* Holmberg

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Sakagami and Zucchi (1974) and Sakagami (1982) have elaborately described the general process of cell construction and oviposition in stingless bees. They distinguished several subdivisions according to the level of integration of the process. Of the genus *Plebeia* only *Pl. minima* (Zucchi, 1977) has been extensively studied, while for some other species some general and incomplete data exist (Imperatriz-Fonseca & Cabral de Oliveira, 1976; Sakagami, 1982). This study refers to the construction and oviposition process of *Pl. remota* Holmberg. Several colonies were used for observations at the University of São Paulo (Brazil).

RESULTS

Cell construction and oviposition in Meliponines is characterized by a varying level of integration. In *Pl. remota* this integration has reached an extremely high level. Synchronisation of the whole process has resulted in a rigid system, as shown in fig.1, of alternating periods of cell construction (extra-oviposition period) and oviposition (oviposition period).

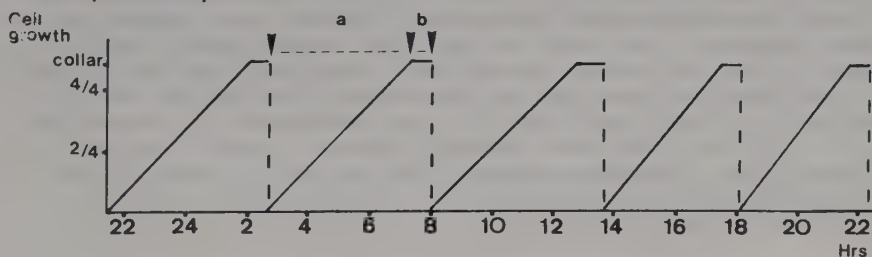


fig.1: progress of cell construction and oviposition.

a = extra-oviposition period; b = oviposition period

The extra-oviposition period is characterized by a relatively low worker excitement in, and by continuous absence of the queen from the construction area. Construction is completely synchronic. At all moments all cells, a "batch", are of identical height. Construction of a batch of cells (formed in horizontal combs) takes 3 to 5.5 hours. The synchronisation of the process is made possible by the disconnection of the traditional bond between cell and worker. The cells are built in a continuous process of successive activities of a group of workers. Each worker of this group was observed to be busy at cells at different sites during one construction cycle.

The oviposition period, starting after completion of a batch of cells, is, in contrast, distinguished by a continuous presence of the queen in the construction area. Her presence is attended by a

general heightening of worker excitement in the area. During this phase, up to the moment of cell provisioning, worker ovipositions may occur. These trophic eggs are slightly larger, with a flexible chorion and more irregular in shape than functional eggs, e.g. as laid by workers in queenless conditions, suggesting an immature state. Oviposition of trophic eggs is rare. They are laid on top of the comb and are usually eaten by the queen, or, when rejected, by a worker.

The general heightening of excitement in the construction area is the first step of an integrated oviposition process (IOP): the traditional unit of the oviposition process, the cell (UOP), is replaced by a batch of cells (n UOP's). Cell provisioning is synchronized. Food discharges occur simultaneously in all cells when the overall excitement has reached a certain level. Each cell is filled by 6 to 9 workers. The queen starts ovipositioning immediately afterwards, without previous cell inspections. After oviposition, a cell is closed instantly by a worker. During this operculation worker ovipositions in the cell, as observed in other stingless bees, were never seen. To underline the integration of the process: in a certain colony an average IOP took 668 sec. ($n=7$), while an average UOP took 488 sec. ($n=21$). A complete oviposition period takes between 25 and 40 minutes.

An interesting aspect of the synchronized provisioning is the appearance of "guards" at the provisioned cells. These guards take position on the outer side of a cell, their head and antennae just above comb level. Since the queen can't visit all cells at the same time, these guards may stay in this position for minutes. The bond between guard and cell is strong: when the queen finally arrives the guard is reluctant to abandon the cell, and has to be pushed away by the queen. The guards were never observed to make any attempt to oviposit in the cell.

Some regulating mechanisms were discerned. The number of cells per batch increased with population size. Batches could number up to 50 cells. Population size didn't influence the rate of construction. However, this rate could be influenced by external conditions. Under flight inhibiting conditions the regular construction rhythm, as shown in fig.1, would slow down to 1 or 2 batches a day, regardless of the colony size. The inflow of nectar seems to be an important regulating factor, since manipulating the colonies by offering sugar solutions partly suppressed the inhibitory effect of unfavourable environmental conditions.

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The Direct Function of Court Behaviour in Stingless Bees

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The occurrence of a royal court with specific queen-worker interactions is characteristic for the intranidal behaviour of stingless bees. For Melipona favosa it was found earlier that court-bees are at the same time the major constructors and provisioners of brood cells. They shuttle frequently between the resting queen - to participate in the court - and the cells under construction. It was hypothesised that the court behaviour of the workers initiates the locomotion of the queen (Sommeijer and De Bruijn, 1984).

MATERIAL AND METHODS

Queen-worker interactions were studied in the following species: Melipona favosa, M. rufiventris, M. trinitatis, Nannotrigona mellaria and Frieseomellita nigra. The detailed behaviour of individually marked court-bees was analysed in order to study the direct function of this behaviour in M. favosa.

RESULTS

The following behavioural acts were recorded: darting, escape, walking away, trophallaxis, crouching, hair-chewing, fixation tapping, tapping frontally, tapping dorsally, rocking movements, backing, following and butting. In all species we recorded: touching the queen by rocking movements, escape, walking away and trophallaxis. Only in Melipona species did we observe hair-chewing, crouching, tapping dorsally, backing and following. In Melipona intrageneric differences were the hair-chewing procedure, crouching and the nature of the dorsal tapping (absent in Melipona favosa). Backing and following were seen only in M. favosa.

The detailed study with marked workers confirmed that court bees are the principal builders and provisioners. The sequential analysis of the various court acts revealed a distinct pattern in the temporal occurrence of certain court acts. For example, 91% of all backing acts (by court workers) were succeeded by the act following (by the queen; n=223). This indicates to what extent certain court-acts of workers initiate the locomotion of the queen. This is further evidence for the communicative function of court-behaviour in stingless bees.

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Chorion Formation in Queen Eggs and Worker Eggs of *Melipona rufiventris*

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The elongated queen egg (QE) has a characteristic surface. The micropyle area is at the anterior pole. The lateral region is characterized by a polygonal network pattern. The posterior region, which in oviposited eggs rests on the surface of the liquid larval food, has no such pattern and is smooth. In a queenright colony, certain workers with activated ovaries deposit eggs in the cell during the provisioning and oviposition process. These worker eggs (WE's), which are normally consumed by the queen, have a reduced micropyle and polygonal network pattern. The micropyle area and the chorion of eggs of queenless workers are similar to those in queen eggs. (Sommeijer et al., 1984).

MATERIALS AND METHODS

Light- and electron-microscopic studies were made of oocytes and recently laid eggs to investigate the deposition of chorion and micropyle material and to study the formation of chorion and micropyle in QE's and WE's.

RESULTS

TEM analysis of eggs and ripening oocytes revealed that the chorion is formed by two types of follicle cells. One type, after migrating between the oocyte and trophocytes, blocks the cytoplasmic bridges and forms the micropyle. The migrated follicle cells form the typical micropylar canals. Both types of follicle cells deposit endochorion material of the vitelline membrane. The exochorion consists of two layers which form the network pattern. The typical local absence of this network is due to the absence of the innermost exochorion layer. The vitelline membrane and the exochorion are absent in the micropyle area.

WE's and QE's have different micropyles. The micropyle of QE's consists of about 90 canals, whereas WE's have fewer than 30 openings. The micropyle-forming follicle-cells deposit electron-dense endochorion around their cytoplasmic projections, thus forming the typical canals. The number of follicle-cells involved determines the size of the micropyle (number of canals).

The absence of the innermost layer of the exochorion (and thus the lack of a network at specific zones) suggests that somewhere in the process of chorion formation the follicle-cells can "skip" a certain phase of secretory activity. Since queenless workers, in contrast to queenright workers, can produce eggs similar to QE's, it is clear that the queen must exert an influence on the development of oocytes in her workers.

Reactions of *Scaptotrigona postica depilis* Colonies to a Change of External Temperature

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Colonial thermoregulation is a widespread phenomenon among social Hymenoptera. Different degrees of regulatory capacity are reported from wasps, bumble bees and honey bees. As little is known about the regulation of nest temperature in stingless bees, we studied reactions to a change of ambient temperature in colonies of *Sc.p.depilis*, a common Brazilian trigonine species nesting in tree cavities and building an involucrum around the brood combs.

MATERIAL AND METHODS

Strong colonies were kept in wooden boxes on the USP campus in Ribeirão Preto in tropical Brazil. Nest temperature measurements were carried out for several weeks during the dry season (Nov. 1985) and the rain period (Jan. 1986), using an electronic device. Small thermosensors were placed in the entrance of the nest, the storage area, inside and outside the layers of involucrum and in the periphery and center of the brood nest. To test thermoregulatory capacity at high ambient temperatures, an observation hive was slowly heated up during a 12 hours period. The behavior of the bees was observed during this shorttime experiment.

RESULTS AND CONCLUSIONS

During the dry and the rainy periods the temperatures at the nest entrance ranged from 18.8°C - 36.0°C. The temperature of the brood nest, however, varied only from 27.5°C - 35.0°C. Depending on ambient temperatures, we could establish a difference of up to 5°C between outside and inside of the involucrum on a distance of only 1 cm. This demonstrates that *Sc.p.depilis* can keep a more or less stable brood nest temperature in cold external conditions. 27°C seems to be the lowest acceptable brood temperature. Warming of the brood chamber is apparently supported by a good isolation capacity of the involucrum.

When we heated up our observation hive from 26°C - 44°C, we recorded brood nest temperatures between 28.7°C and 39.6°C. During the experiment we hardly observed any typical cooling behavior of the bees, although on other occasions fanning occurred. At brood nest temperature of 35°C as a first reaction the movements of the bees on the brood combs got much faster. When brood temperature reached 36°C, the bees became strongly excited and a large number left the brood combs. At 38°C and more hardly any bee remained in the brood chamber. In the entrance tube many bees were excitedly running in and out with expanded wings. At the nearby ponds no water collecting bees were observed. In the morning after the experiment the situation within the hive appeared to be normal again.

Therefore, we conclude that cooling capacity in this species is only little developed. In the natural tropical environment heating of the brood nest seems to be more important than cooling it. Brood warming is achieved by many workers crowding the combs. Protection against high ambient temperatures may be facilitated also by selection of shady nesting locations. -Supported by a DAAD grant.

Relations between Eye Structure and Mating Biology in the Stingless Bee, *Scaptotrigona postica depilis*, and the Honey Bee, *Apis mellifera carnica*

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In many bees mating biology includes visual detection of the female by the male. Olfactory stimuli, i.e. the female sex pheromone, may attract the male as well. In a comparative study of reproductive biology in the highly eusocial stingless bees and honey bees we analyzed the relative significance of both types of cues in the allocation of a virgin queen by the drones. Here we report on morph-specific eye structures.

MATERIALS AND METHODS

Compound eye cornea replicas were prepared by spraying clear gloss varnish and after removal flattened, microphotographed and measured. Cornea thickness was determined using toluidine stained araldite cross sections. Eye micrographs were taken from sputtered whole mounts with a Cambridge Stereoscan 250 MX2 electron microscope.

RESULTS

In the Brazilian stingless bee, *Scaptotrigona postica depilis*, the compound eyes of drones, queens and workers are of a similar size, consisting of about 4.000 ommatidia. Facet diameters vary in the range of approximately 10 to 20 μm , and cornea thickness between 30 and 50 μm , with minor mean values in the queens. In the honey bee, *Apis mellifera carnica*, the compound eyes of queens and workers do not differ much. Both have a little less than 5.000 ommatidia. The honey bee drone eye, however, is greatly enlarged. The surface area is more than twice that of the female castes, consisting of approximately 10.000 ommatidia. The drone compound eyes extend on the dorsal side of the head, where the rims touch each other. The range of facet diameters in drones (about 20 - 30 μm) exceeds that of workers and queens (about 15 - 20 μm). The same is true for cornea thickness (male 55 - 65 μm versus female 25 - 40 μm). Within the morphs of the two species compared here, the honey bee drone has a compound eye exceptional in size and structural specialization (Fig. 1).

In bee compound eyes the area of largest facets is normally found around the center, extending mainly somewhat to the ventral side. This intraocular distribution of large facets which provide enhanced visual acuity, is realized especially in the worker honey bee. The resulting view field probably is used for flower detection during foraging. In the queen honey bee and also in all the three morphs of the stingless bee a similar arrangement of different sized corneal facets is found. Again the drone honey bee is an exception. Only in this morph is the large facet area located exclusively in the dorsal part of the compound eye. The corresponding field of vision is directed upwards-forwards. Therefore, a sexual dimorphism of compound eye structures is only pronounced in honey bees, not in stingless bees.

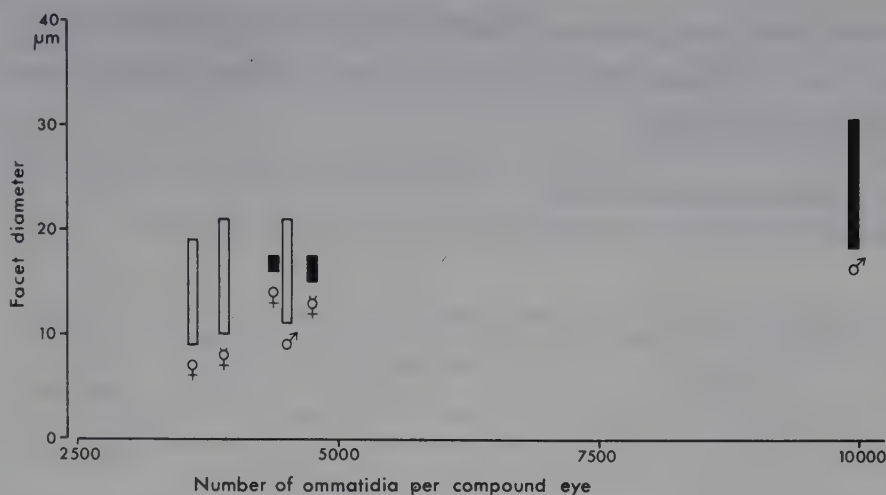


Fig. 1. -- Relationship between number and size range of ommatidia in the compound eye of the three morphs in the stingless bee (open bars) and the honey bee (solid bars). Notice the exceptional number of large ommatidia in the drone honey bee.

DISCUSSION

These remarkable structural peculiarities of the drone honey bee compound eye must be of functional significance. Of course the enlarged eye surface enables the drone to recognize subjects in extreme lateral and dorso-frontal positions. As large facets provide an enhanced visual acuity, the target for detection by the giant facets is supposedly in front of the drone and in a position superior to the longitudinal body axis. As mating is the most important event in the life of an adult drone, the peculiar compound eye structure is probably of significance for detection of a queen during the nuptial flight. Drones are known to chase queens only during a rapid flight over a drone congregation area. Queen location against the bright sky high over the vegetation indeed must require specialized structural and functional adaptations of the visual system. The morphology of the honey bee drone compound eye as described here is interpreted to be an optimized detection apparatus for such targets as a chased queen.

On the contrary stingless bees mainly mate in the shadow of the dense vegetation of the tropical rain forest. Drone aggregations are formed in the vicinity of nests containing virgin queens. In this environment the males can probably better locate a queen on a nuptial flight using olfactory cues, i.e. pheromonal signals. Therefore, in stingless bee drones no special eye structures like those of the honey bee are found.

It can be concluded from this comparative study that special drone eye structures only developed in the honey bee in relation to a specific mating biology - especially the visual detection of the queen during the nuptial flight - whereas in stingless bees the drone apparently locates the queen by means of pheromones and, therefore, does not need an evolved visual detection system.

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Nectar Dehydration and Body Weight Reduction: the Cues of *Xylocopa nigrocincta* to Prolong Duration of Territorial Flights

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X. nigrocincta males are nest constant. During the mating periods (October–November; January–March) the males are fed nectar by the oldest bees. During the day males dehydrate the nectar at the nest entrance. For that they regurgitate a droplet and amplify its surface area by repeatedly spreading it between the galeae, thus improving water evaporation. In the late afternoon males fly out on non resource based territories in trees, where they spend up to two hours flying in circles of about 2 m Ø. Asking for the function of nectar dehydration by *X. nigrocincta* males, two hypotheses were tested:

1. Males could feed back the dehydrated nectar to foragers. These would then fly out with high sugar concentration in low nectar volume. The capacity of their honeycrops for collected nectar would be high. Per trip they could collect more nectar.

2. By dehydrating nectar, males reduce its volume and therefore can receive further nectar quantities which in total – and without dehydration – would exceed the capacity of their honeycrop. Thus they could enrich the energy (sugar) content of their nectar loads. By evaporating excessive water, males could reduce the weight of the nectar they carry. Thus they could minimize their energy expenses during territorial flights.

MATERIALS AND METHODS

To test whether males distribute nectar to nest mates 165 ml nectar of 45% sugar concentration (wt/wt) were artificially stained (food stain) and fed to males and in a second experiment to females of *Xylocopa nigrocincta* nests. After 6, 12 and 48h all bees of the nests were forced to regurgitate and the nectar was checked for coloring.

To test the second hypothesis, 18 nests of *X. nigrocincta* were observed during the mating periods from 6.00h – 19.00h. The sugar concentration of returning foragers was determined with a field refractometer. The honeycrops of 25 males leaving and returning from territorial flights were depleted and nectar quantity and its sugar concentration were determined. The nectar then was fed back to the males. Time was recorded from the moment the males were released till they returned from territorial flights. From g sugar/ml nectar the energy content (Joule) was calculated (Bolten et al., 1979). Energy was plotted against flight duration.

RESULTS

1. The distribution of stained nectar between *X. nigrocincta* nest mates revealed that males do not feed back the nectar to females or other males. Therefore the first hypothesis can be rejected.

2. *X. nigrocincta* foragers collected nectar of a mean sugar concentration of 29% (range: 18% – 34.8%). When leaving the nest males had

dehydrated the nectar to a mean sugar concentration of 57%. The maximum dehydration rate measured was 40.5% (from 26.5 - 67%). Honeycrops of males flying out on territories contain between 75 μ l and 107 μ l nectar (\bar{x} = 39 ml), although the total holding capacity of their honeycrops is about 165 μ l. Depending on the energy content of the nectar the males total flight duration ranged between 20 min. and 177 min. Energy content of nectar and duration of flight were positively correlated (Fig.1).

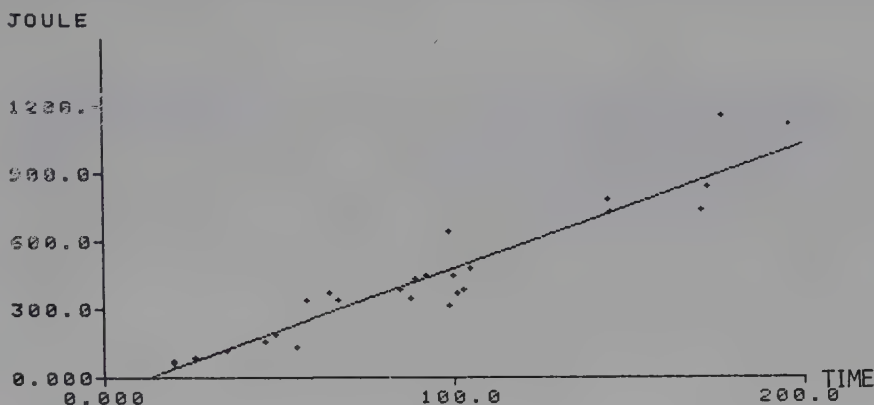


Fig. 1. -- Correlation between the energy (Joule) the males carried and time spent on territorial flights.

Of males which had equal energy resources when leaving the nest, those which carried less water, i.e. which dehydrated their nectar to higher sugar concentrations, spent more time on territorial flights.

DISCUSSION

For all male Hymenoptera (and others) which establish and defend territories, energy consumption during territorial flights is a crucial problem. Even more so, if the territory is a non resource based one, which males have to leave to take up energy at nectar plants. In the time absent they might miss females or lose their territory to invaders. Thus the time spent in a territory should be correlated with mating success. *X. nigrocincta* males match this problem by dehydrating nectar at the nest before they fly out on territories. We conclude that all effects of nectar dehydration such as volume reduction (which makes enrichment of energy by multiple nectar uptake possible) and reduction of weight (which improves the energy budget during flight) work together to prolong the time a male can spend in a territory. Good dehydrators thus may be favoured by sexual selection. We have good evidence that males of at least one other *Xylocopa* species also dehydrate nectar. We suppose that further studies will reveal similar pre-mating behavior in other bee genera with territorial males.

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Bionomy of Five Xylocopine Bee Species in Rio Grande do Sul, Southern Brazil

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During a three year bee survey in Rio Grande do Sul eleven xylocopine bee species were found, two of them not yet described for this state. In October 1984, several nests of *X. frontalis*, *X. nigrocincta*, *X. macrops*, *X. varians varians* and *X. augusti* were collected in Porto Alegre or nearby. The nests were continuously observed from October 1985 until March 1986. The bees were marked individually with enamel paints. Seasonal fluctuation of daily activity of foraging bees and male seasonality and mating behavior were studied. Furthermore the durations of larval development, nest architecture and substrat preference for nest construction were investigated. A special point of interest were the social interactions amongst the nestmates in the overlapping periods between two generations.

All these species are wood dwellers but differences in nest site preference and nest architecture favor their sympatric occurrence. The five observed species have similar durations of larval development and two generations per year: the winter generation emerges in March/April, but stays inactive till reproductive phase starts in September/October, the summer generation emerges in late December/January and starts reproductive activity in January/February.

Daily activity shows seasonal fluctuations depending on onset of daylight, temperature and weather conditions. The females observed in the summer have a high peak of flight activity in the early morning. In the afternoon a second small peak occurs around 14.00h. In the winter, daily activity is completely different. The females start flight activity at approximately 20 °C. This minimum take-off temperature is reached during winter days about noon. In winter flight activity caeses in the early afternoon, whereas in summer the bees were active till sunset. Maternal and filial generations overlap temporarily. In this temporarily eusocial period social interactions occur between mothers and offspring or amongst the offspring (sister-sister and sister-brother interactions). Normally the mother provisions the offspring with nectar and pollen until the nestmates leave the nest or a daughter starts to forage. In case of absence of the mother, sisters provision brothers and/or sisters. Females and sometimes also males defend the nest against intruders, especially ants. One or more sisters may stay in the birth nest and construct their own nest, branching from the old nest, or may leave the nest to start their own. Male mating behavior of the observed species is different. Males of *X. nigrocincta* return to their home nest after territorial flights, while others like *X. frontalis* males abandon their home nests after a period of transition or even after the first flights (*X. varians varians*). Males staying in the birth nest have the advantage, that they are fed by female nestmates and don't risk their lives by leaving the nest for self-provisioning flights. At the end of the mating season males of these species are not longer accepted in their home nests by mothers or sisters. Males abandoning their nests provision themselves and also have to discover locations to stay. Often males of different nests of the same species gather in male groups which inhabit abandoned nests (*X. frontalis*, *X. varians varians*). Two different forms of mating in territories in xylocopine bees were observed. Males either fly in non resource based territories attracting females with pheromones (*X. frontalis*, *X. nigrocincta*), or wait for females in territories above food plants (*X. macrops*).

Caste and Sex Morphometry in *Bombus atratus* in Comparison to *Apis* and *Melipona*

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The Mahalanobis distance - as a generalized biometric mean value based on a number of morphometric characters - has been calculated for the three morphs occurring in bees: queen, worker, and drone. Published data refer to the honey bee, *Apis mellifera*, represented by the Africanized bee now common in Brazil, and three species of stingless bees of the genus *Melipona*. In *Apis*, the queen-to-worker distance (0.6) is shorter than the distances between the two female castes and the drones. In contrast, the respective data for *Melipona compressipes* are 1.8, for *M. quadrifasciata* 1.1, and for *M. scutellaris* 1.7. This longer distance means that, in stingless bees, workers resemble drones more than queens. The same situation is described here for a neotropical bumble bee, *Bombus atratus*. All these data are used here to reconstruct phylogenetic relationships between Apinae, Meliponinae, and Bombinae. The latter are perhaps more closely related. Possible involvements of juvenile hormone in caste differentiation are discussed.

Caste Differentiation in Stingless Bees: Activity of *Corpora Allata* and Ecdysteroid Titters during the Critical Last Larval Instar

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The essential role of juvenile hormone in the control of caste differentiation has been demonstrated in a considerable number of species of the genus *Melipona* and for several trigonine genera. In all these studies JH or JH-analogues had been applied topically to last instar worker larvae, and, despite the well known differences in the mechanisms of caste determination between *Melipona* and the trigonine genera, a period of sensitivity to exogenous JH was generally found in cocoon spinning larvae.

As opposed to the other two groups of eusocial bees, the genera - *Apis* and *Bombus* - hardly anything has been known so far on the endogenous hormonal basis of caste differentiation in stingless bees. For this reason an attempt was made to follow the cycles of JH synthesis activity in the corpora allata (CA), and to determine the haemolymph titers of ecdysteroids during the last larval instar (L5) for the two castes of the stingless bee species *Scaptotrigona depilis*.

MATERIALS AND METHODS

The activity of JH synthesis in the CA of *Scaptotrigona depilis* worker and queen larvae was measured by a radiochemical assay developed by Pratt and Tobe (1974). 6-8 pairs of corpora allata with annexed corpora cardiaca and pars intercerebralis had to be used per incubation. They were incubated in modified honey bee medium (Kaatz et al., 1985).

Haemolymph titers of ecdysteroids were measured by a RIA with an ICT-antibody (Spindler et al., 1978).

RESULTS

Fig. 1 gives a compilation of the measured rates of JH synthesis in the CA, and of ecdysteroid titers in the haemolymph of queen and worker larvae of *Scaptotrigona depilis* during successive stages of the last larval instar.

Since queen larvae are significantly larger than worker larvae, body weight values were used as correction factors to exclude the possibility of the observed differences in CA activities simply resulting from differences in body size. However, even after these corrections queen CA proved to be distinctly more active than worker CA throughout the whole last larval instar. In order to detect caste specific differences in the timing of CA activities queen/worker ratios were calculated for the corrected JH synthesis rates. While during most part of L5 the CA activity ratio gave values between 1.2 and 1.9, a queen specific peak of differential CA activity became apparent at the transition from the feeding to the spinning phase, i.e. shortly before the onset of a - for *S. depilis* though still hypothetical - JH sensitive period for the induction of queen development.

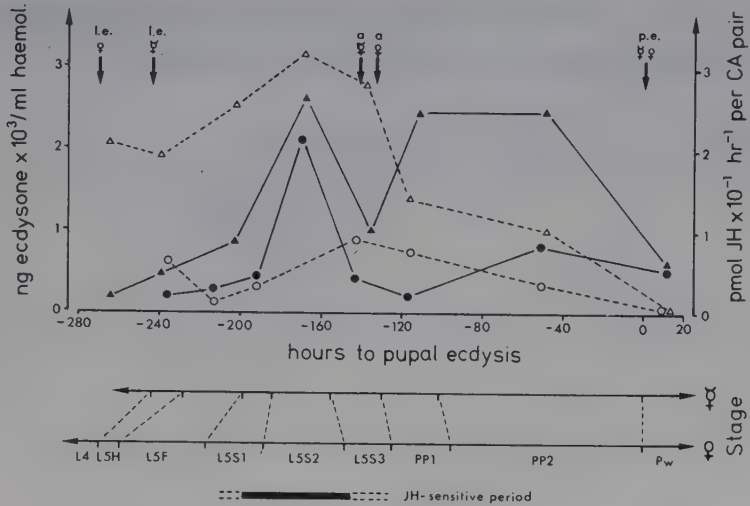


Fig. 1: CA activity (----) and ecdysteroid titer (—) in the last larval instar of *S. depilis* workers (○---○ and ●—●) and queens (△---△ and ▲—▲) drawn on an absolute time scale. The duration of the single stages is given below, as well as the position of the caste relevant JH-sensitive period (determined for *Scaptotrigona xanthotricha* by Campos et al., 1983). a, apolysis, l.e., larval ecdysis; p.e. pupal ecdysis.

DISCUSSION

The pattern of fluctuations in the ecdysteroid titers and the cycles in CA activity during the last larval instar of *S. depilis* queens and workers are consistent with our present day knowledge and general models on metamorphosis in holometabolous insects (Riddiford, 1980). The functional significance of each of these hormone peaks in the regulation of metamorphosis events, however, is known in detail only for lepidopteran and dipteran species. With respect to bees we largely have to rely on analogies with the lepidopteran model systems. Thus we are in the dilemma that we cannot yet assign a function to the caste specific modulation of ecdysteroid titers which have now become apparent as a common theme in all highly eusocial groups of bees. (Hagenguth & Rembold, 1978; Strambi et al., 1984).

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Occurrence and Distributional Limits of Euglossine Bees in Rio Grande do Sul, Southern Brazil

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Euglossine bees are restricted to the neotropics where they reach from Northern Mexico to Northern Argentina (Moure, 1964). Euglossine females collect nectar and pollen from a few plant species, males collect fragrances from orchids and other plants. To which extent food plant abundance and the availability of scent sources determine the distribution of euglossine bees was studied during a survey of the so far unknown euglossine bee fauna in Rio Grande do Sul, Southern Brazil.

MATERIALS AND METHODS

From December 1984 till March 1985 euglossine males were censused by attracting them to cineole, vanillin and scatole baits on four transects which cover the main vegetational, geomorphological and climatic units of Rio Grande do Sul. Three forest types contribute to the floral composition of this State: 1. The Araucaria forest, which provides only few potential food and fragrance sources for euglossine bees, 2. The subtropical rain forest and 3. the Atlantic rain forest in which at least 93 plant species, which are potential nectar and pollen plants for Euglossini, are common. Of these species 90% occur in the Atlantic rain forest and 67% in the subtropical rain forest. Of the plants, except orchids, from which males collect fragrances, at least 31 species occur, 93% of them in the Atlantic- and 31% in the subtropical rain forest. The abundance of orchids is augmented in the Atlantic rain forest, where 97% of the species occur. In contrast only 6% are common in the subtropical rain forest (Rambo, 1961).

RESULTS

Five euglossine bee species, among them two new species, were found (numbers give rel. abundance): Eufriesea (Ef.) violacea (97.8%), Eulaema (El.) nigrita (1.1%), Euglossa (Eg.) sp. nov. (0.31%), Eg. cordata (0.31%) and Ef. sp. nov. (0.16%). The dominant species rank of Ef. violacea was consistent at all study sites. In the subtropical rain forest Ef. violacea, El. nigrita, Eg. cordata and Eg. sp. nov. were found. In this habitat species richness and absolute abundance diminishes on a north-south gradient. On the E-W transect, which covers the region where the subtropical- and the Atlantic rain forest intermingle, absolute abundance of euglossine bees increased saltatory in the Atlantic rain forest. In the Atlantic rain forest 3 euglossine bee species were recorded: Ef. violacea, El. nigrita and Ef. sp. nov. In this forest type the absolute abundance of euglossine bees is about nine times higher than in the subtropical rain forest. At the eastern rim of the highlands, where the Atlantic rain forest overlaps with the Araucaria forest, Ef. violacea and El. nigrita were found. Further inland the Araucaria forest no more euglossine bees visited the baits. In the south of the State, Ef. violacea and Eg. cordata were found in an

isolated habitat of the subtropical rain forest. South of about 30°S no more Euglossini were recorded (Fig.1).

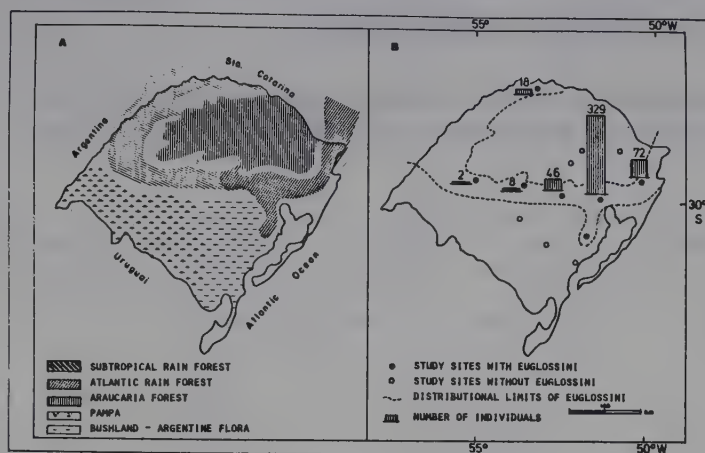


Fig.1. -- A. Phytogeography and B. Distribution of euglossine bees in Rio Grande do Sul

DISCUSSION

Low diversity and abundance of food plants and fragrance sources make the Araucaria forest an unfavourable habitat for euglossine bees. Altitude and climatic features of the highlands are subordinate factors, as euglossine bees were found in the highest and coldest part of the highlands, where they were restricted to the Atlantic rain forest, but did not enter the adjacent Araucaria forest. In the subtropical rain forest the lack of orchids and the overall low resource availability in comparison to the Atlantic rain forest does limit the habitat to small numbers of euglossine bees. Their abundance increases where the habitat characteristics are changed by the influence of the Atlantic rain forest. The ample supply of resources for euglossine bees, which is strongly expressed in orchid species and individual abundance, makes the Atlantic rain forest the most favourable habitat for euglossine bees in Rio Grande do Sul. As an overall pattern of euglossine bee distribution in Southern Brazil we find western and eastern populations which are separated by the habitat of the Araucaria forest. Only at the southern rim of the highlands, where the Araucaria forest ends, these populations are connected by a narrow "East-West Passage", which is the southern limit of euglossine bee distribution in Brazil.

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Seasonality and Seasonal Changes in Preferences for Chemical Baits of Male *Eufriesea violacea* in Rio Grande do Sul, Southern Brazil

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Euglossine bees collect floral fragrances from a variety of plant families, especially from orchids of the subtribes Catasetinae and Stanhopeinae and from genera like *Aspasia*, *Cattleya*, *Maxillaria*, *Rodriguezia*, *Zygopetalum* etc. (Dodson, 1966; Zucchi et al., 1969). Explanations for the fragrance collecting behavior are still hypothetical. The fragrances may serve as precursors for sex pheromones. Many of these fragrances were isolated from orchids (Williams and Whitten, 1983) and can be used as baits to attract euglossine males. During an initial survey of euglossine bees in Rio Grande do Sul, Southern Brazil, five euglossine bee species were recorded of which *Eufriesea violacea* is the most abundant one. Here we describe its seasonality and give a first record of seasonal changes in preferences of euglossine bees for chemical fragrances.

MATERIALS AND METHODS

From December 1984 till March 1985 the seasonal abundance of male euglossine bees was monitored in an Atlantic rain forest habitat, by attracting the bees to vanillin, cineole and scatole from 9.00h till 13.00h. The baits were kept under permanent observation and each male arriving was noted. After a first evidence for seasonal changes in preferences of males for the fragrances, the study was repeated at the same site in 1985/86. Numbers of males collecting fragrances from the baits were recorded 5 times throughout the season with repetitions on three consecutive days.

RESULTS

In both years the flight activity of male *Eufriesea violacea* lasted about 90 days. The abundance profiles are of an identical "big bang" type, but the population peaked about 30 days earlier in 1985/86 than in the year before (Fig.1). In 1984/85 male activity at the baits reached its maximum between 11.00 h and 12.00 h, while in 1985 / 86 males were more active during the morning. Consistently for both years the preference of *Eufriesea violacea* males for

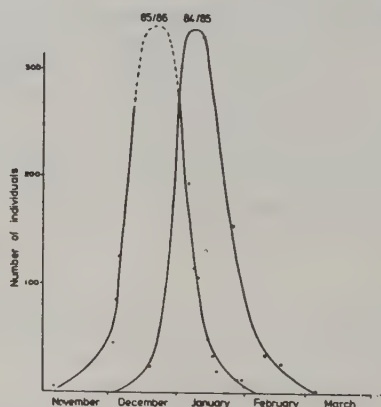


Fig.1. -- Abundance profiles of populations of *Eufriesea violacea* males in the Atlantic rain forest of Rio Grande do Sul.

single chemical fragrances changed throughout the season (Fig.2).

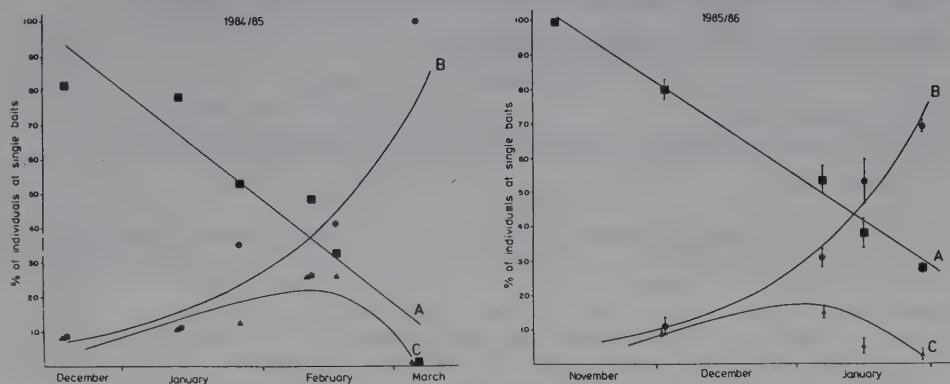


Fig.2. -- Seasonal changes in preferences for chemical fragrances in Eufriesea violacea males. A: vanillin, B: cineole, C: scatole

At the beginning of the season males had a high preference for vanillin, which decreased linearly throughout the season, while the preference for cineole increased exponentially. The attractivity of scatole was constantly low and ceased at about the time when cineole became more attractive than vanillin.

DISCUSSION

Euglossine bees of the genus Eufriesea are distributed from Mexico to Southern Brazil (Moore, 1967). Many species of this genus are known to occur at chemical baits for comparatively short periods of a few months (Ackermann, 1982). The short flight activity of Eufriesea violacea may favour its dominant abundance in the Brazilian subtropics, where the climate is distinctly seasonal. The extremely cold and rainy winter in 1984 may have retarded the onset of the population development in the following season. However, population sizes were not influenced by the climatic conditions of the two years. The changes in the preferences of Eufriesea violacea males for vanillin, cineole and scatole may reflect changes in the abundance of naturally occurring scent sources. Final causal explanations may be given, when the function of male fragrance collecting is definitely understood.

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Hypothesis Generation and Testing in Ethnobiology and Ethnoentomology: the "Intellectual Bridge" between Sciences and Cultures

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The interdisciplinary Kayapo project has developed methodological procedures to scientifically test hypotheses generated through its ethnobiological investigations of indigenous ecological knowledge (Posey, 1986). Native concepts and beliefs are used by Western scientists as *emic* (internal or indigenous) guides for their research designs (Posey, 1983a, 1985). Data collection utilizes indigenous categories for biological inventories, while ecological concepts (often couched in myth and natural symbols) establish the basis for interdisciplinary dialogue and research. In this manner, indigenous knowledge of biological communities and ecological relationships can be studied; when non-Western notions arise, these are formulated as hypotheses and tested by respective specialists.

RESULTS

For example, nine new species of stingless bees (Meliponinae) were discovered through the comparison of Kayapo and Western taxonomic systems (Posey, 1983b). The utility of Indian knowledge about bee behavior in the development of studies in areas little known to entomologists, such as: differences in odor characteristics, swarming behavior, flight patterns, and habitat choices between or within meliponine species was recorded by Posey and Camargo (1985). They also propose scientific investigation based on indigenous knowledge of bee species distribution in different ecological zones and habitat sharing by certain species clusters. Indian ideas of acrid commensalism and use of odor trails by species for which such activity is yet unreported have also spurred further studies by entomologists of stingless bee behavior.

A large inventory of arthropod agricultural pests based on Indian information and confirmed by field collections was effected by Overall and Posey (1986). They also report the development of research into the highly effective control of agricultural pests in indigenous gardens through intercropping, use of trap crops and natural predators. The Indians attribute much of this natural control to predatory ants, wasps, and termites all of which are glorified in Kayapo myth and song. Roles of these insects in crop pest control are currently being investigated following indigenous guidelines.

The Kayapo utilize *Azteca* spp. ants to repel leaf-cutting "sauva" (*Atta* spp.) (Kerr and Posey, 1984). Likewise, Kerr and Posey (1986) report the indigenous use of several natural pesticides and call for their testing by Western sciences. At least in the case of *Azteca* spp., very positive results from scientific tests to determine their effectiveness in the protection of Amazonian citrus were published (Overall and Posey, 1986).

The intentional planting of certain floral species by Indians to attract bees was observed (Posey, 1983, Anderson and Posey, 1985). Such knowledge can be helpful in the investigation of tropical pollination and aid in the improvement of apiculture.

Many bee species are thought by the Kayapo to have important medicinal properties (Posey, 1983b). Such properties are practically unknown by pharmacologists and need to be investigated for their effectiveness and

potential for a natural pharmacopeia (Elizabetsky and Posey, 1987).

These are but a few examples from a single ethnobiological project of how indigenous knowledge can stimulate new ideas for Western sciences. No researcher is expected to accept prima facie all native believes. Much indigenous knowledge, as we have already seen, is highly symbolic and difficult for even the most experienced researcher to interpret; however, nothing can be dismissed by the ethnobiologists no matter how ridiculous it may initially sound. The most ludicrous ideas today may offer the greatest insights tomorrow when their symbols are finally decoded.

Refusal by Western scientists to study native believes is, after all, not a very scientific attitude. It is much more scientific to test the validity of native observations through the testing of hypotheses generated by ethnobiological study.

CONCLUDING REMARKS

Folk systems of knowledge have in most cases developed for many millenia and are frequently more ancient than Western science. They reflect the diversity of ways in which the natural world can be ordered, and provide detailed information of ethology, ecological communities, useful species, and biological diversity. Folk knowledge can also serve to generate new ideas and hypotheses that can be investigated and tested with the rigors of occidental science. Studies of folk knowledge as outlined in this paper offer a powerful "intellectual bridge" between different peoples. Understanding the science of other cultures enriches Western science and provides the philosophical bases for the understanding and appreciation of other peoples on and in their own terms.

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ABSTRACTS

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